



Ahfad University for Women
School of Health Sciences
MS Program in Nutrition and Dietetic

Advanced Human Nutrition: Foundations

*Reference Reader for the
MS Program in Nutrition
and Dietetics — AUW 2026*

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About this Reader

This comprehensive reader, titled "**Advanced Human Nutrition Reader for Nutrition and Dietetic Master Program**," is authored by **Professor Osama Awad Salih** for the School of Health Sciences at **Ahfad University for Women**. It serves as a foundational resource for the MS Program in Nutrition and Dietetics, specifically designed to bridge the gap between advanced nutritional theory and clinical application.

The reader is organized into four distinct modules that cover the essential pillars of modern nutritional science:

- **Module 1: Micronutrients & Protective Elements** – Deep dives into vitamins, minerals, and the biochemical mechanisms of phytonutrients and antioxidants.
- **Module 2: Body Regulation & Composition** – Examines internal balance through fluid/electrolyte regulation and the assessment of body composition throughout the human lifespan.
- **Module 3: Nutritional Assessment & Specialized Needs** – Focuses on evaluating dietary intake and managing nutritional requirements for specific groups, including pregnant women, infants, and the elderly.
- **Module 4: Oxidative Stress, Phytochemicals & Functional Foods** – Explores the role of functional foods and phytochemicals in chronic disease prevention and the management of oxidative stress.

N.B.

Note on Supplementary Material:

Following this reader is a specialized **Clinical Supplement** that provides an in-depth focus on the **Clinical Reasoning Approach**. This supplement explores the science of protective plant compounds and the mechanics of bioavailability and dietary interactions. It guides the practitioner through the "Why" and "How" of nutrition: why deficiencies happen, who is at risk, and how dietitians respond using the ADIME framework and advanced biomarkers. Additionally, it provides expert clinical prescriptions for energy expenditure, hunger regulation, acid-base balance, and the management of sarcopenia and adiposity.

How to Use this Reader?

To maximize your learning from this graduate-level series, it is recommended that students and practitioners engage with the material using the following structured approach:

1. **Review Learning Outcomes First:** Each lecture begins with specific "Learning Outcomes." Use these as a checklist to ensure you can describe biochemical pathways, interpret clinical markers, and apply evidence-based reasoning by the end of your study session.
2. **Focus on Clinical Integration:** Pay close attention to the "Clinical Note" and "Clinical Pearl" sections. These are designed to help you apply theoretical knowledge—such as mineral-mineral interactions or fat malabsorption—to real-world patient scenarios.
3. **Utilize the "Lecture Summaries":** At the end of major sections, refer to the "Key Principles" summaries. These consolidate complex information into manageable takeaways, such as the specific metabolic niches of B vitamins or the factors affecting mineral bioavailability.
4. **Engage with "Practice" Recommendations:** Use the practical counseling tips, such as "Eat the Rainbow" or the specific preparation methods for cruciferous vegetables, to transform academic data into actionable dietary advice for patients.
5. **Consult Suggested Readings:** For deeper exploration of specific topics (e.g., the hepcidin/ferroportin axis or specific DRI reports), use the curated list of suggested academic texts and research papers provided at the end of each lecture.

Advanced Human Nutrition: Foundations

A Reference Reader for the MS Program in Nutrition and Dietetics

Professor Osama Awad Salih | MS Program Lecture Series No. 1 | School of Health Sciences, AUW 2026

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This Reader provides the scientific basis, biochemical mechanisms, and evidence-based content that constitutes the intellectual framework of the Advanced Human Nutrition course. Each lecture follows a structured academic format including Learning Outcomes, biochemical foundations, clinical notes, DRI reference values, and suggested readings. For clinical application, case-based reasoning, and dietetic practice guidance, see the companion volume: Advanced Human Nutrition: Clinical Reasoning — A Practice-Oriented Companion for the MS Program in Nutrition and Dietetics.

Module 1

Micronutrients & Protective Elements

Professor Osama Awad Salih

NUTRITION & DIETETICS
MS Program Lecture Series

Micronutrients & Protective Elements

A 3-Lecture Graduate Series

Covering: Vitamins • Minerals • Phytonutrients & Antioxidants

Professor Osama Awad Salih

Lecture 1 Fat-Soluble Vitamins A • D • E • K	Lecture 2 Water-Soluble Vitamins B-Complex • Vitamin C	Lecture 3 Minerals & Phytonutrients
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LECTURE 1

Fat-Soluble Vitamins

Vitamins A, D, E & K — Biochemistry, Metabolism & Clinical Applications

Nutrition & Dietetics MS Program | Department of Nutritional Sciences

Learning Outcomes — By the end of this lecture, students will be able to:

- Describe the biochemical structure and metabolic pathways of vitamins A, D, E, and K
- Explain absorption mechanisms via chylomicrons and hepatic/extrahepatic distribution
- Correlate deficiency and toxicity states with underlying mechanisms
- Interpret clinical and biochemical assessment markers for each vitamin
- Apply evidence-based reasoning to supplementation decisions in clinical practice

I. FOUNDATIONS: FAT-SOLUBLE VITAMINS

A. Defining Characteristics & Common Properties

Fat-soluble vitamins (FSV) — A, D, E, and K — share several key properties that differentiate them from their water-soluble counterparts and have significant clinical implications:

- Solubility & absorption: Require dietary fat and bile salt emulsification for intestinal absorption; incorporated into micelles and subsequently into chylomicrons
- Transport: Circulate bound to specific carrier proteins (retinol-binding protein, vitamin D-binding protein) or lipoproteins
- Storage: Stored predominantly in the liver and adipose tissue; significant reservoir capacity
- Elimination: Excreted via bile and feces, NOT urine — this contributes to accumulation and toxicity risk
- Toxicity risk: Chronic high-dose supplementation can cause hypervitaminosis (most notable for A and D)
- Deficiency timeline: Depletion occurs slowly due to tissue stores; clinical deficiency may not manifest for months

Assessment note: Because FSVs are stored in tissues, serum levels may not accurately reflect true body stores in all contexts. Functional biomarkers (e.g., prothrombin time for K, night blindness for A) may be more clinically informative.

II. VITAMIN A (Retinoids & Carotenoids)

A. Chemical Forms & Sources

Vitamin A encompasses a family of compounds with retinol activity:

- Preformed vitamin A (retinoids): Retinol, retinaldehyde (retinal), retinoic acid — found in animal foods (liver, dairy, eggs, fish)
- Provitamin A carotenoids: β -carotene (most potent), α -carotene, β -cryptoxanthin — found in plant foods (orange/yellow/dark green vegetables); converted to retinal in enterocytes
 - Conversion efficiency: $1\text{ }\mu\text{g retinol} = 12\text{ }\mu\text{g }\beta\text{-carotene} = 24\text{ }\mu\text{g other provitamin A carotenoids}$ (Retinol Activity Equivalents, RAE)
 - Conversion is regulated by vitamin A status — prevents toxicity from plant sources

B. Absorption & Metabolism

Dietary retinyl esters $\rightarrow$ hydrolysis by pancreatic retinyl ester hydrolase $\rightarrow$ retinol $\rightarrow$ re-esterified in enterocytes $\rightarrow$ packaged into chylomicrons $\rightarrow$ lymphatic transport $\rightarrow$ liver (hepatic stellate cells store as retinyl esters; hepatocytes release retinol bound to RBP4). Target tissues receive retinol via RBP4-STRA6 receptor or from circulating retinyl esters in lipoproteins.

C. Biochemical Functions

1. Vision

Retinal (11-cis) binds opsin in rod cells to form rhodopsin. Photon absorption isomerizes to all-trans retinal $\rightarrow$ initiates photo transduction cascade $\rightarrow$ nerve impulse. All-trans retinal is recycled via the visual cycle (requires RPE65 enzyme). Depletion of retinal in rod outer segments $\rightarrow$ night blindness (nyctalopia), the earliest deficiency symptom.

2. Gene Transcription — Nuclear Receptor Signaling

Retinoic acid (RA) is the most transcriptionally active form. RA binds Retinoic Acid Receptors (RAR $\alpha/\beta/\gamma$) and Retinoid X Receptors (RXR $\alpha/\beta/\gamma$), forming heterodimers that bind Retinoic Acid Response Elements (RAREs) in gene promoters. This regulates expression of >500 genes involved in:

- Cellular differentiation & morphogenesis (embryogenesis, epithelial cell maturation)
- Immune function (T-cell differentiation, IgA production, mucosal immunity)
- Hematopoiesis, spermatogenesis, and osteoblast/osteoclast activity

3. Antioxidant Function (Carotenoids)

β -carotene and other carotenoids function as antioxidants through singlet oxygen quenching and free radical scavenging. Protective associations have been observed epidemiologically against cancer and CVD, though supplementation trials (ATBC, CARET) showed increased lung cancer risk in smokers, suggesting context-dependency of antioxidant supplementation.

D. Deficiency: Vitamin A Deficiency (VAD)

VAD is the leading preventable cause of childhood blindness globally; a major public health issue in Sub-Saharan Africa and South/Southeast Asia.

Clinical Manifestation	Underlying Mechanism
Night blindness (nyctalopia)	↓ rhodopsin synthesis due to retinal depletion
Xerophthalmia (Bitot's spots, corneal ulceration)	Squamous metaplasia of conjunctival/corneal epithelium
Follicular hyperkeratosis	Impaired epithelial differentiation → keratin plug formation
Increased infection susceptibility	Compromised mucosal barriers; impaired T-cell & NK cell function
Anemia (functionally)	↓ mobilization of iron from stores; may mimic iron deficiency anemia
Impaired growth	Disrupted IGF-1 signaling; reduced cell proliferation

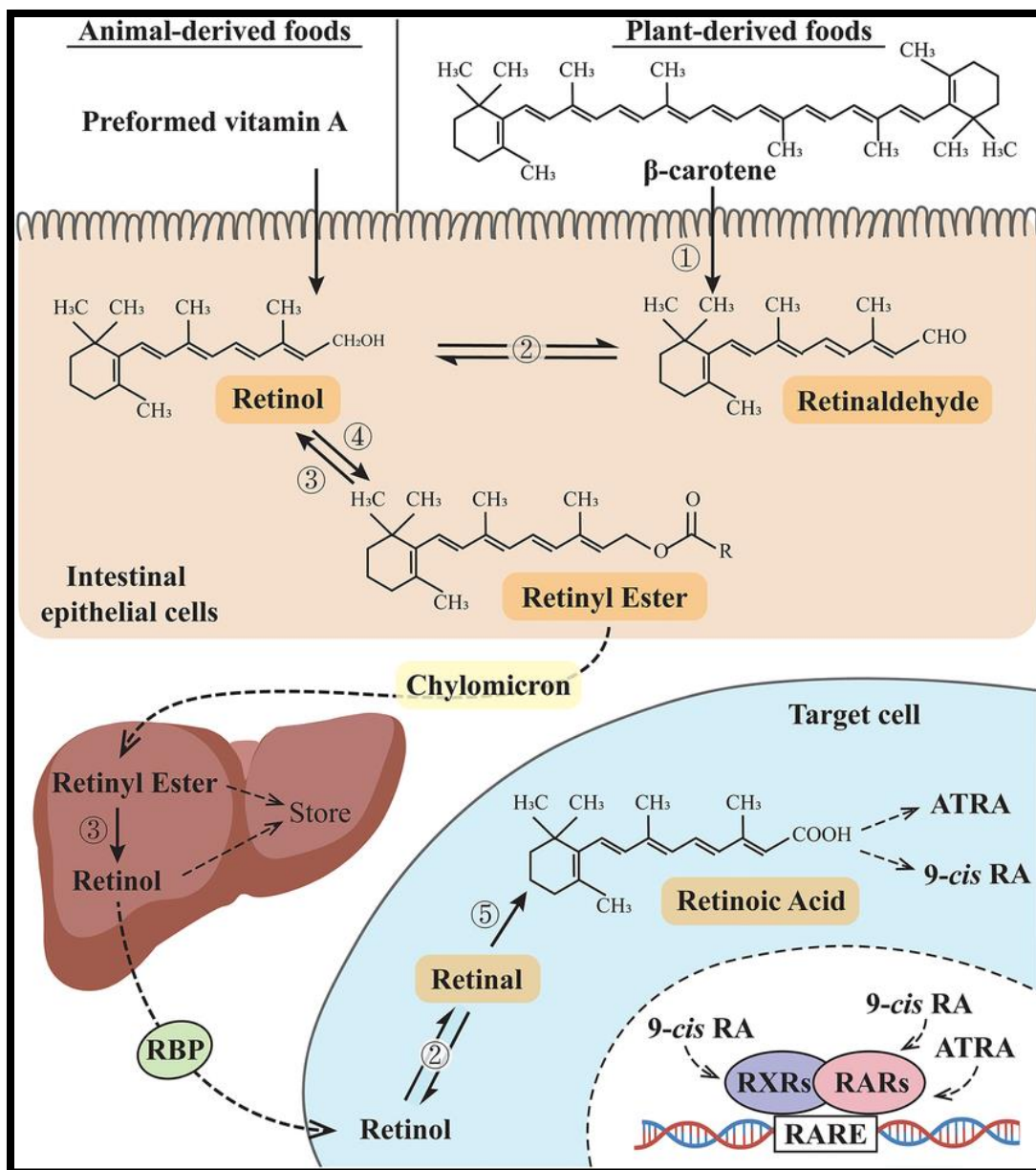
E. Toxicity: Hypervitaminosis A

Clinical Note: Toxicity occurs only from preformed vitamin A (retinoids), NOT from dietary carotenoids. At-risk groups include: individuals on high-dose supplements, those with liver disease, and infants receiving megadose supplementation.

- Acute (>150,000 µg/day): Nausea, headache, increased intracranial pressure (pseudotumor cerebri)
- Chronic (>10,000 µg/day over months): Hepatotoxicity, hyperlipidemia, alopecia, bone pain, teratogenicity
- Teratogenicity: Retinoic acid at high doses → craniofacial, cardiac, and CNS malformations; UL in pregnancy = 3,000 µg RAE/day
- Tolerable Upper Intake Level (UL): 3,000 µg RAE/day (adults)

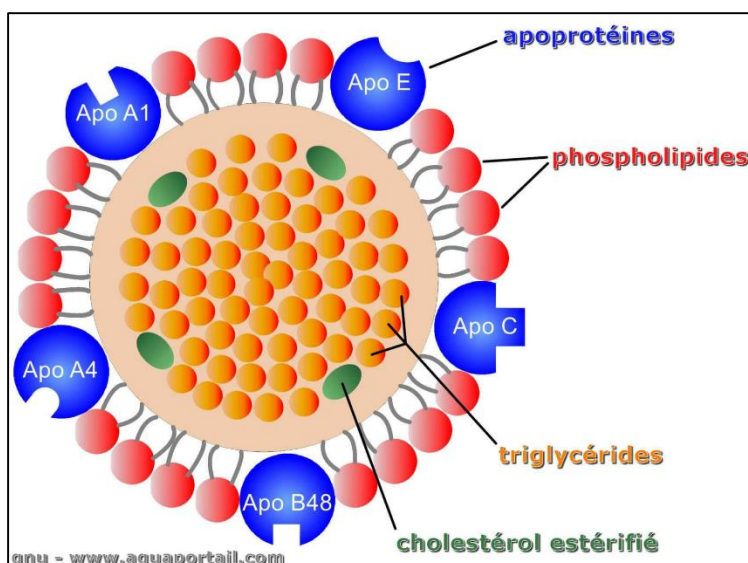
F. DRI Reference Values & Assessment

RDA (adults): 700 µg RAE (women) / 900 µg RAE (men). Assessment: Serum retinol (reflects status when stores severely depleted; insensitive until stores very low), modified relative dose response (MRDR) test, plasma retinol binding protein.



Vitamin A metabolic pathway.

- ① β -Carotene 15,15'-dioxygenases;
- ② Retinol dehydrogenases (RDHs): RDH1, RDH10, and short-chain dehydrogenase/reductase (SDR family) member 9 (DHRS9) (reversible reaction);
- ③ retinyl esterase;
- ④ lecithin: retinol acyltransferase (LRAT) or acyl-CoA: retinol acyltransferase (ARAT);
- ⑤ retinaldehyde dehydrogenases (RALDH).
- RBP, retinol-binding protein; 9-cis RA: 9-cis retinoic acid; ATRA, all-trans retinoic acid; RXRs, retinoid X receptors; RARs, retinoic acid receptors; RARE, retinoic acid response elements.



Chylomicrons are large, triglyceride-rich lipoprotein particles synthesized in the intestine to transport dietary lipids (fats and cholesterol) from the digestive tract to tissues throughout the body. **also known as ultra low-density lipoproteins (ULDL)**. Formed in enterocytes, they carry over 75% triglyceride content, enabling absorption of dietary fat into the bloodstream via the lymphatic system.

Key Aspects of Chylomicrons:

- **Composition & Function:** They are composed of triglycerides (83–92%), phospholipids, cholesterol, and a specific protein called Apolipoprotein B-48. They transport dietary fat to peripheral tissues (adipose, muscle) for energy or storage.
- **Production & Transport:** After a fatty meal, they appear in the blood within 1 hour, delivering nutrients before being reduced to remnants

Clearance: Lipoprotein lipase (LPL) breaks down the triglycerides, while the liver removes the remaining "chylomicron remnants".

- **Clinical Significance:** High levels of chylomicrons indicate high triglycerides, which can lead to complications such as pancreatitis, fatty deposits under the skin, or indicate metabolic disorders like diabetes.

Lipoproteins are particles made of protein and lipids (fats) that transport cholesterol and triglycerides through the bloodstream to cells. They consist of a hydrophobic core surrounded by a hydrophilic shell. Major types include HDL (good cholesterol), LDL (bad cholesterol), and VLDL, which are critical for lipid metabolism.

Types of Lipoproteins

1. **HDL (High-Density Lipoprotein):** Known as "good" cholesterol, it carries cholesterol from tissues back to the liver to be removed.
 2. **LDL (Low-Density Lipoprotein):** Known as "bad" cholesterol, high levels can build up in blood vessel walls, causing plaque and increasing heart disease risk.
 3. **VLDL (Very Low-Density Lipoprotein):** Primarily carries triglycerides to tissues.
 4. **Lipoprotein(a) [Lp(a)]:** A specific type of LDL that, when high, significantly increases the risk of heart attack, stroke, and arterial disease, often determined by genetics.
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Structure and Function

- **Composition:** They are assembled from triglycerides, cholesterol esters, and phospholipids, with embedded proteins called apolipoproteins.
 - **Transportation:** Because fats cannot dissolve in water (blood), these particles act as "molecular trucks" to move lipids throughout the body.
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Clinical Significance

- **Heart Health:** High LDL and low HDL levels are major risk factors for cardiovascular disease.
 - **Testing:** Lipoprotein (a) is often tested to assess independent risk for heart disease, especially if there is a family history.
 - **Management:** Lifestyle changes (diet, exercise) can lower LDL, while high Lp(a) levels are largely genetic and are the subject of new, targeted therapies
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III. VITAMIN D (Calciferols)

A. Forms, Sources & Synthesis

- Vitamin D2 (ergocalciferol): Plant/fungal origin (UV-irradiated ergosterol); found in mushrooms, supplements
- Vitamin D3 (cholecalciferol): Animal origin + endogenous skin synthesis; more potent at raising 25(OH)D levels
- Cutaneous synthesis: UVB (290–315 nm) converts 7-dehydrocholesterol → pre-vitamin D3 → vitamin D3. Affected by skin pigmentation, latitude, season, sunscreen, age (↓ efficiency)

B. Activation (Two-Step Hydroxylation)

Vitamin D3 → 25-hydroxycholecalciferol [25(OH)D, calcidiol] via hepatic 25-hydroxylase (CYP2R1) → 1,25-dihydroxycholecalciferol [1,25(OH)₂ D, calcitriol] via renal 1α-hydroxylase (CYP27B1). This is the hormonally active form. Renal 1α-hydroxylase is tightly regulated by PTH (stimulates), FGF-23 (inhibits), calcium, and phosphate. Extra-renal 1α-hydroxylase activity exists in macrophages, skin, placenta — important for local immune and cellular effects.

C. Mechanisms of Action

1. Classical — Calcium & Bone Homeostasis

Calcitriol binds Vitamin D Receptor (VDR) → VDR: RXR heterodimer → binds Vitamin D Response Elements (VDREs) → transcription of:

- Intestinal calbindin-D9k/D28k: Calcium-binding proteins; facilitate transcellular Ca²⁺ absorption
- TRPV6 & PMCA1b: Apical Ca²⁺ channel and basolateral Ca²⁺ pump in enterocytes
- Renal calcium reabsorption proteins in distal tubule
- RANKL expression in osteoblasts → promotes osteoclast differentiation → bone resorption when Ca²⁺ levels low

2. Non-Classical Actions — Emerging Evidence

- Immune modulation: Promotes tolerogenic dendritic cells, Tregs; inhibits Th1/Th17; may protect against autoimmune diseases
- Muscle function: VDR in skeletal muscle; deficiency → proximal myopathy, increased fall risk
- Cardiovascular: VDR in cardiomyocytes and vascular smooth muscle; epidemiological links to hypertension and CVD
- Anti-proliferative/pro-differentiation: VDR activation in cancer cells → cell cycle arrest; basis for oncology research
- Mental health: Brain VDR expression; associations with depression, schizophrenia, cognitive decline

D. Deficiency

Clinical Note: Vitamin D deficiency is a global pandemic — estimated >1 billion people worldwide have deficiency or insufficiency. At-risk groups: elderly, dark-skinned individuals in northern latitudes, exclusively breastfed infants, those with fat malabsorption syndromes, obesity (sequestration in adipose tissue).

- Rickets (children): Failure of bone mineralization → soft, deformable bones; genu varum/valgum, rachitic rosary, craniotables, delayed fontanelle closure
- Osteomalacia (adults): Painful bone disorder; proximal muscle weakness; pseudo fractures (Looser zones on imaging)
- Secondary hyperparathyroidism: $\downarrow \text{Ca}^{2+} \rightarrow \uparrow \text{PTH} \rightarrow$ bone resorption → contributes to osteoporosis
- Increased fracture risk, sarcopenia, falls (particularly in elderly)

E. Assessment & DRI Values

Primary marker: Serum 25(OH)D (reflects total vitamin D status from all sources). Classification: Deficiency <30 nmol/L (<12 ng/mL); Insufficiency 30–49 nmol/L (12–19 ng/mL); Sufficiency ≥50 nmol/L (≥20 ng/mL); Optimal (some experts): 75–100 nmol/L. Toxicity risk >250 nmol/L.

DRI: RDA = 600 IU/day (ages 1–70), 800 IU/day (>70 years). UL = 4,000 IU/day (adults). Note: Many experts argue RDA is insufficient to achieve optimal serum levels in most populations without significant sun exposure.

IV. VITAMIN E (Tocopherols & Tocotrienols)

A. Chemical Forms & Bioactivity

Vitamin E includes 8 naturally occurring congeners: α -, β -, γ -, δ -tocopherols and corresponding Tocotrienols (differ in side chain saturation). α -Tocopherol is the most biologically active and is the form maintained in plasma by hepatic α -tocopherol transfer protein (α -TTP). γ -Tocopherol (prevalent in Western diet) is preferentially catabolized. Major food sources: vegetable oils, wheat germ, nuts/seeds, green leafy vegetables.

B. Primary Function: Chain-Breaking Antioxidant

α -Tocopherol is the most important lipid-soluble antioxidant in biological membranes. It donates a hydrogen atom to lipid peroxyl radicals ($\text{LOO}\cdot$) → forming a tocopheroxyl radical ($\text{Toc}\cdot$) → stops chain propagation of lipid peroxidation. The $\text{Toc}\cdot$ is then reduced (regenerated) by vitamin C (ascorbate) at the membrane-aqueous interface — illustrating nutrient antioxidant network synergy. This action is critical for protecting:

- Polyunsaturated fatty acids (PUFAs) in cell membranes
- LDL particles from oxidation (oxidized LDL is central to atherogenesis)
- Mitochondrial and ER membranes in metabolically active tissues

C. Additional Roles

- Enzyme regulation: Inhibits PKC; modulates platelet aggregation and smooth muscle proliferation
- Gene expression: Regulates expression of connective tissue proteins, liver enzymes; α -TTP has a role in lipid metabolism beyond vitamin E transport
- Anti-inflammatory: Inhibits arachidonic acid metabolism; suppresses NF- κ B pathway at high doses

D. Deficiency & Assessment

Primary dietary deficiency is rare in healthy adults due to abundant food sources and efficiency of storage. Deficiency occurs in: fat malabsorption syndromes (cystic fibrosis, cholestatic liver disease, abetalipoproteinemia), premature infants (low adipose stores), and genetic α -TTP deficiency (rare, causes spinocerebellar ataxia).

- Neurological manifestations: Peripheral neuropathy (loss of deep tendon reflexes $\rightarrow$ ataxia), spinocerebellar degeneration, ophthalmoplegia
- Hemolytic anemia: In premature infants — RBC membrane fragility due to lipid peroxidation

Clinical Note: Assessment: Serum α -tocopherol (ideally expressed per mg total lipid to correct for hyperlipidemia); erythrocyte hemolysis test. RDA = 15 mg α -TE/day. UL = 1,000 mg/day (from supplements — risk of hemorrhage via anticoagulant effects).

V. VITAMIN K (Phylloquinones & Menaquinones)

A. Forms, Sources & Intestinal Microbiome Role

- Vitamin K1 (Phylloquinones, K1): Predominant dietary form; green leafy vegetables, plant oils; primarily taken up by liver
- Vitamin K2 (Menaquinones, MK-4 to MK-13): Produced by gut bacteria (MK-7 most abundant bacterially derived form); found in fermented foods (natto, aged cheese); longer half-life; more bioavailable to extrahepatic tissues
- Gut microbiome contribution: Colonic bacteria synthesize MKs, but colonic absorption is limited — dietary sources remain primary; however, intestinal dysbiosis or prolonged antibiotic use can worsen K status

B. Mechanism of Action: γ -Carboxylation

Vitamin K functions as a cofactor for the enzyme γ -glutamyl carboxylase. This enzyme converts specific glutamate (Glu) residues to γ -carboxyglutamate (Gla) residues in vitamin K-dependent proteins. The Gla residues chelate Ca^{2+} ions, enabling these proteins to bind phospholipid membranes and become biologically active. Vitamin K is consumed in this reaction (converted to vitamin K epoxide) and regenerated by VKOR (Vitamin K Epoxide Reductase) — the target of warfarin anticoagulation.

C. Vitamin K-Dependent Proteins & Their Functions

Protein	Function & Clinical Relevance
Factors II, VII, IX, X (coagulation)	Pro-coagulant cascade; K deficiency → hemorrhagic disease; warfarin effect
Proteins C, S, Z (anticoagulation)	Endogenous anticoagulants — paradoxically, acute K deficiency may initially impair these before pro-coagulant factors
Osteocalcin (bone)	Binds calcium in bone matrix (hydroxyapatite); carboxylated form correlates with bone density
Matrix Gla Protein (MGP)	Potent inhibitor of vascular and soft tissue calcification; under-carboxylated MGP = calcification risk
Gas6	Growth arrest-specific gene 6; involved in cell proliferation, survival, inflammation resolution
Periostin, GRP	Extracellular matrix organization; emerging roles in inflammation and kidney function

D. Deficiency, Drug Interactions & Assessment

Clinical Note: Warfarin (coumadin) inhibits VKOR → prevents vitamin K recycling → functionally induces K deficiency in the coagulation pathway. Monitor INR carefully. Consistent dietary K1 intake is more important than avoidance when on warfarin.

- Deficiency risk: Prolonged antibiotic therapy, fat malabsorption, neonates (universal vitamin K prophylaxis at birth: VKDB prevention), liver disease
- Hemorrhagic disease of the newborn (VKDB): Breast milk has low vitamin K; neonatal gut microbiome not established → fatal bleeding risk without prophylaxis
- Assessment: Prothrombin time (PT/INR) for coagulation; plasma Phylloquinones; under-carboxylated osteocalcin (ucOC) and PIVKA-II as functional markers
- AI (not RDA — insufficient data): 90 µg/day (women), 120 µg/day (men); No established UL for food-derived K

VI. CLINICAL INTEGRATION & KEY TAKEAWAYS

Lecture 1 Summary — Key Principles

- Fat-soluble vitamins share bile-dependent absorption; stored in liver/adipose; eliminated via bile (↑ toxicity risk for A and D)
- Vitamin A: Essential for vision (rhodopsin), gene transcription (RAR/RXR), immune epithelial integrity; VAD = preventable blindness; toxicity is teratogenic
- Vitamin D: A prohormone requiring two-step hydroxylation; primary function = calcium homeostasis; emerging immunomodulatory and metabolic roles; global deficiency pandemic
- Vitamin E: Primary membrane antioxidant; chain-breaking lipid peroxidation terminator; regenerated by vitamin C — illustrates antioxidant network
- Vitamin K: γ-Carboxylation cofactor; critical for coagulation, bone (osteocalcin) and vascular health (MGP); K2 menaquinones have important extrahepatic roles
- Clinical pearl: Fat malabsorption (IBD, bariatric surgery, cystic fibrosis, cholestatic liver disease) → monitor ALL fat-soluble vitamins

Suggested Reading:

1. Combs GF & McClung JP (2022). The Vitamins, 5th Ed. Chapters on A, D, E, K. |
2. Ross AC et al. Modern Nutrition in Health and Disease, 11th Ed. | Institute of Medicine DRI Reports.

LECTURE 2

Water-Soluble Vitamins

B-Complex Vitamins & Vitamin C — Coenzyme Biochemistry, One-Carbon Metabolism & Antioxidant Roles

Nutrition & Dietetics MS Program | Department of Nutritional Sciences

Learning Outcomes — By the end of this lecture, students will be able to:

- Identify the coenzyme form of each B vitamin and describe its metabolic role
- Trace the one-carbon metabolism pathway and explain the roles of folate, B12, and B6
- Connect homocysteine metabolism to cardiovascular and neurological disease risk
- Describe vitamin C's role in collagen synthesis and as an antioxidant
- Differentiate deficiency presentations and identify high-risk clinical populations

I. OVERVIEW: WATER-SOLUBLE VITAMINS

Water-soluble vitamins (WSV) encompass the eight B vitamins and vitamin C. Key shared properties:

- Absorbed directly into portal circulation (no chylomicron packaging required)
- Minimal storage (exception: B12 — significant hepatic stores; biotin partly stored)
- Excess excreted renally — generally lower toxicity risk from food sources; however, pharmacological doses of B3, B6, and C can cause adverse effects
- Most function as coenzyme precursors — they are biologically inert until converted to their active coenzyme forms
- Deficiency develops relatively quickly (weeks to months), especially with high metabolic demand

II. B VITAMINS — COENZYME FUNCTIONS & METABOLISM

A. Thiamin (Vitamin B1)

Active coenzyme form: Thiamin pyrophosphate (TPP). Dietary sources: whole grains, legumes, pork, nutritional yeast. RDA: 1.1–1.2 mg/day.

Biochemical roles of TPP:

- Pyruvate dehydrogenase complex (PDC): Pyruvate → Acetyl-CoA (glycolysis → TCA cycle junction — critical for aerobic glucose oxidation)
- α -Ketoglutarate dehydrogenase: TCA cycle (α -KG → Succinyl-CoA)
- Transketolase: Pentose phosphate pathway (PPP) — nucleotide synthesis & NADPH production
- Branched-chain α -keto acid dehydrogenase: BCAA catabolism

Clinical Note: Brain and nervous tissue are highly dependent on glucose metabolism via these pathways — explaining the neurological predominance of thiamin deficiency. Neurons cannot store thiamin; even short-term deficiency causes dysfunction.

Deficiency Syndromes:

- Dry Beriberi: Peripheral neuropathy — symmetric distal sensory loss, muscle weakness, decreased reflexes
- Wet Beriberi: High-output heart failure (↑ cardiac demand + impaired energy metabolism) with peripheral edema
- Wernicke-Korsakoff Syndrome (WKS): Acute Wernicke's encephalopathy (ophthalmoplegia, ataxia, confusion) → chronic Korsakoff psychosis (anterograde amnesia, confabulation); precipitated by alcohol use disorder, often triggered by IV glucose without thiamin supplementation
- Refeeding syndrome risk: Glucose infusion in depleted patients rapidly consumes thiamin reserves → acute WE

B. Riboflavin (Vitamin B2)

Active coenzyme forms: Flavin Mononucleotide (FMN) and Flavin Adenine Dinucleotide (FAD). Sources: dairy, meat, eggs, green vegetables, fortified grains. RDA: 1.1–1.3 mg/day.

FAD/FMN serve as prosthetic groups of flavoenzymes, acting as electron carriers (accept/donate 2H via redox cycling):

- Electron transport chain (Complex I, II): Core role in mitochondrial ATP synthesis
- β -Oxidation: Fatty acyl-CoA dehydrogenase (MCAD, LCAD, SCAD) — each round of β -ox produces 1 FADH₂
- TCA cycle: Succinate dehydrogenase (Complex II)
- Activation of other B vitamins: Required for conversion of B6 → PLP, folate → active forms, and niacin synthesis from tryptophan

Clinical Note: Riboflavin deficiency (Ariboflavinosis) is often subclinical. Manifestations: angular cheilitis, glossitis (magenta tongue), seborrheic dermatitis, normochromic anemia. Critically, riboflavin deficiency impairs the function of ALL other B vitamins — making it a gateway nutrient deficiency.

C. Niacin (Vitamin B3)

Active coenzyme forms: NAD⁺ (Nicotinamide Adenine Dinucleotide) and NADP⁺ (Nicotinamide Adenine Dinucleotide Phosphate). Sources: meat, poultry, fish, peanuts, mushrooms, fortified grains. Can also be endogenously synthesized from tryptophan (60 mg Trp = 1 mg niacin equivalent). RDA: 14–16 NE/day.

NAD⁺ /NADH and NADP⁺ /NADPH are the most abundant redox coenzymes in cells, involved in >500 enzymatic reactions:

- NAD⁺ (oxidized): Accepts H⁻ in catabolism — glycolysis, TCA cycle, β -oxidation → drives ATP synthesis via ETC
- NADP⁺ : Anabolic reactions — fatty acid synthesis (FAS), cholesterol synthesis; NADPH from PPP
- NAD⁺ as signaling substrate: Sirtuin deacetylases (SIRT1–7), PARP (DNA repair), CD38 (Ca²⁺ signaling) all consume NAD⁺ — linking niacin/NAD⁺ to aging, epigenetics, and DNA integrity

Clinical Note: Pellagra (the 4 Ds): Dermatitis (photosensitive, Casal necklace), Diarrhea, Dementia, Death. Historically linked to maize-dominant diets (maize niacin bound as niacytin; tryptophan also limiting). Still seen in alcoholism, Hartnup disease, carcinoid syndrome. Pharmacological niacin (1.5–3 g/day) raises HDL and lowers LDL/TG but causes flushing (prostaglandin-mediated, COX-1 dependent).

D. Pantothenic Acid (Vitamin B5)

Active form: Coenzyme A (CoA) and acyl carrier protein (ACP). Found in virtually all foods (most abundant in organ meats, eggs, whole grains); deficiency is exceedingly rare. RDA: AI = 5 mg/day.

- CoA: Central carrier for acyl groups in fatty acid synthesis, β -oxidation, TCA (acetyl-CoA, succinyl-CoA), and amino acid catabolism
- ACP: Prosthetic group of fatty acid synthase (FAS) complex; carries growing acyl chain during de novo lipogenesis
- 'Burning feet syndrome': Rare deficiency (experimental) — paresthesia, malaise; reversible

E. Pyridoxine (Vitamin B6)

Active coenzyme form: Pyridoxal-5-phosphate (PLP). Sources: meat, fish, poultry, potatoes, bananas, chickpeas. RDA: 1.3–1.7 mg/day.

PLP is an extraordinarily versatile coenzyme, acting as an electrophilic catalyst in >100 enzymatic reactions involving amino acid metabolism:

- Transamination: Transfer of amino groups between amino acids and α -keto acids (AST, ALT — used as liver function markers)
- Decarboxylation: Production of biogenic amines — serotonin (from 5-HTP), dopamine (from DOPA), GABA (from glutamate), histamine (from histidine), epinephrine/norepinephrine
- Glycogen phosphorylase: Glycogenolysis (allosteric modulation by PLP)
- One-carbon metabolism: Serine hydroxymethyltransferase (SHMT) — serine $\rightarrow$ glycine + 5,10-methylene-THF; interacts with folate cycle
- Heme synthesis: Aminolevulinic acid synthase (ALA synthase) — rate-limiting step
- Homocysteine metabolism: Cystathionine β -synthase (CBS) — transsulfuration of homocysteine $\rightarrow$ cystathionine

Clinical Note: *B6 deficiency $\rightarrow$ impaired neurotransmitter synthesis $\rightarrow$ irritability, depression, peripheral neuropathy. Also causes sideroblastic anemia (impaired heme synthesis) and seborrheic dermatitis. UL = 100 mg/day; peripheral sensory neuropathy observed at chronic doses >200–500 mg/day ('megadose neuropathy').*

F. Biotin (Vitamin B7)

Active form: Biotin covalently attached to carboxylase enzymes via holocarboxylase synthetase. Sources: eggs (cooked), liver, nuts, legumes; gut bacteria contribute. AI = 30 μ g/day.

Biotin is the essential cofactor of the four biotin-dependent carboxylase enzymes:

- Pyruvate carboxylase (PC): Pyruvate $\rightarrow$ Oxaloacetate (gluconeogenesis, anaplerosis)
- Acetyl-CoA carboxylase (ACC1/2): Acetyl-CoA $\rightarrow$ Malonyl-CoA (rate-limiting step in fatty acid synthesis; also regulates β -oxidation)
- Propionyl-CoA carboxylase (PCC): Propionyl-CoA $\rightarrow$ Methylmalonyl-CoA (odd-chain FA, branched chain AA catabolism)
- 3-Methylcrotonyl-CoA carboxylase (MCC): Leucine catabolism

Clinical Note: *Raw egg white contains avidin — a glycoprotein that tightly binds biotin ($K_d \sim 10^{-15}$ M) and prevents absorption. Regular consumption of many raw eggs can cause biotin deficiency: hair loss (alopecia), dermatitis, neurological symptoms. Cooking denatures avidin. Biotinidase deficiency: genetic disorder — impairs recycling of biotin from protein catabolism; neonatal screening.*

G. Folate (Vitamin B9)

Active coenzyme form: Tetrahydrofolate (THF) and its various one-carbon substituted derivatives (5-methyl-THF, 5,10-methylene-THF, 10-formyl-THF). Dietary folate: polyglutamate forms in food → hydrolyzed by intestinal folate conjugase → monoglutamate for absorption. Folic acid (synthetic): monoglutamate → higher bioavailability (~1.7x dietary folate). Sources: green leafy vegetables (foliage = folate), legumes, liver, fortified grains. RDA: 400 µg DFE/day; 600 µg DFE pregnancy.

Folate's critical roles in one-carbon (1C) metabolism:

- Nucleotide synthesis: 5,10-methylene-THF → dTMP (thymidylate synthase); 10-formyl-THF → purine ring carbons — essential for DNA replication and repair
- Methylation reactions: 5-methyl-THF donates methyl group to B12 (methionine synthase) → regenerates methionine → SAM synthesis → >200 methylation reactions (DNA, RNA, histones, neurotransmitters, phospholipids)

Clinical Note: *Megaloblastic (macrocytic) anemia: Folate deficiency → impaired dTMP synthesis → impaired DNA replication → abnormally large RBC precursors (megaloblasts); ineffective erythropoiesis. MTHFR polymorphisms (C677T, A1298C): Reduce 5-methyl-THF synthesis; ↑ homocysteine; associated with ↑ risk of NTDs, CVD, certain cancers; may require higher folate intake. Neural tube defects (NTDs): Folate-dependent → periconceptional supplementation (400–800 µg/day) reduces NTD risk by ~70%.*

H. Cobalamin (Vitamin B12)

Active coenzyme forms: Methylcobalamin (methyl-B12) and Adenosylcobalamin (AdoB12). Sources: exclusively animal products (meat, fish, dairy, eggs) — synthesized only by microorganisms. Vegans require supplementation. RDA: 2.4 µg/day.

Absorption (highly complex):

Dietary B12 → released from food proteins by gastric acid + pepsin → binds R-protein (haptocorrin) in stomach → R-protein cleaved by pancreatic proteases → B12 binds Intrinsic Factor (IF, secreted by gastric parietal cells) → IF-B12 complex absorbed by cubilin receptor in terminal ileum. Defect at any step causes deficiency.

Biochemical functions:

- Methionine synthase (MS) — Methylcobalamin: Homocysteine + 5-methyl-THF → Methionine + THF. This regenerates methionine for SAM synthesis AND releases active THF for nucleotide synthesis. B12 deficiency → folate 'methyl trap' (folate locked as 5-methyl-THF; cannot be used for DNA synthesis) → megaloblastic anemia
- L-Methylmalonyl-CoA mutase — Adenosylcobalamin: L-methylmalonyl-CoA → Succinyl-CoA (mitochondrial, part of odd-chain FA and BCAA catabolism). Deficiency → methylmalonic acidemia → toxic to myelin

Clinical Note: *Neurological manifestations of B12 deficiency (subacute combined degeneration of the spinal cord): Demyelination of dorsal and lateral columns → paresthesias, proprioception loss, ataxia, spastic paraparesis. These neurological symptoms can be MASKED if folate is supplemented without addressing B12 deficiency — a critical clinical distinction.*

- Pernicious anemia: Autoimmune destruction of parietal cells → ↓ IF → B12 malabsorption; requires parenteral or high-dose oral B12 (passive absorption)
- Assessment: Serum B12 (insensitive at low-normal levels); methylmalonic acid (MMA) and homocysteine are more sensitive functional markers of depletion

III. ONE-CARBON METABOLISM — INTEGRATED PATHWAY

One-carbon (1C) metabolism is a network of interconnected reactions that transfer single-carbon units, essential for nucleotide synthesis, methylation, and redox balance. The key nutrients are folate, B12, B6, riboflavin, choline, betaine, and methionine.

Step / Enzyme	Nutrients Required
Serine → Glycine + 5,10-methylene-THF (SHMT)	Folate (THF), PLP (B6)
5,10-methylene-THF → 5-methyl-THF (MTHFR)	Folate, FAD (B2) as cofactor
5-methyl-THF + Homocysteine → Methionine (MS)	B12 (methylcobalamin)
Methionine → SAM → SAH → Homocysteine	Methionine (dietary), many methyltransferases
Homocysteine → Cystathionine (CBS)	PLP (B6)
Homocysteine → Methionine via betaine-HCy methyltransferase	Betaine / Choline

Elevated homocysteine (hyperhomocysteinemia) is a biomarker of 1C metabolism dysfunction and is independently associated with increased CVD risk, venous thromboembolism, and cognitive decline/Alzheimer's disease risk. Causes: B12, folate, or B6 deficiency; MTHFR polymorphisms; renal insufficiency (↓ clearance); hypothyroidism.

IV. VITAMIN C (L-Ascorbic Acid)

A. Chemistry & Sources

Vitamin C is a water-soluble lactone with a powerful reducing capacity ($E^{\circ} = +0.077$ V at physiological pH). Most animals synthesize ascorbic acid from glucose (via L-gulonolactone oxidase); humans, other primates, guinea pigs, and fruit bats lack this enzyme due to GULO pseudogene. Dietary sources: citrus fruits, kiwi, strawberries, bell peppers (richest), broccoli, tomatoes. RDA: 75 mg/day (women), 90 mg/day (men); +35 mg for smokers (oxidative stress ↑ turnover). UL = 2,000 mg/day.

B. Biochemical Functions

1. Collagen Synthesis — Prolyl & Lysyl Hydroxylation

Vitamin C is an essential cofactor for prolyl hydroxylase (PH) and lysyl hydroxylase (LH) — iron-dependent dioxygenases that hydroxylate proline and lysine residues in procollagen chains. Hydroxyproline stabilizes the collagen triple helix (hydrogen bonding); hydroxylysine enables crosslink formation via lysyl oxidase. In ascorbate deficiency, un-hydroxylated collagen chains are unstable → do not form proper triple helices → degraded → weakened connective tissue → scurvy.

Clinical Note: Scurvy: Perifollicular hemorrhages (corkscrew hairs), bleeding gums (gingivitis), poor wound healing, hemarthrosis, periosteal hemorrhage. Historically a disease of sailors/explorers (6–8 weeks without dietary vitamin C). Now seen in elderly, food-insecure populations, and those with severely restrictive diets. Rapidly reversed by ascorbic acid supplementation.

2. Carnitine Synthesis

Two ascorbate-dependent hydroxylation steps in carnitine biosynthesis (from lysine and methionine): trimethyllysine hydroxylase and γ -butyrobetaine hydroxylase. Carnitine deficiency contributes to the muscle fatigue of scurvy.

3. Iron Absorption Enhancement

Ascorbate reduces Fe^{3+} (non-heme iron) to Fe^{2+} in the intestinal lumen (Fe^{2+} is transported by DMT-1; Fe^{3+} is not). Ascorbate also chelates iron, keeping it soluble in the alkaline duodenum. Co-ingestion of 50–100 mg vitamin C with a meal significantly increases non-heme iron bioavailability (2–4 fold) — of major clinical relevance for vegetarians/vegans.

4. Antioxidant Function

Ascorbate is the primary aqueous-phase antioxidant. It scavenges reactive oxygen species (ROS): superoxide ($\text{O}_2^{\bullet-}$), hydroxyl radical ($\bullet\text{OH}$), singlet oxygen ($^1\text{O}_2$), hypochlorous acid (HOCl). Ascorbate reduces the tocopheroxyl radical (Toc $\bullet$) at the membrane-aqueous interface $\rightarrow$ regenerates α -tocopherol — a key example of the antioxidant network. In plasma, ascorbate protects LDL from oxidative modification.

5. Additional Roles

- Norepinephrine synthesis: Dopamine β -hydroxylase (copper-dependent; ascorbate as reductant)
- Neuropeptide amidation: Peptidylglycine α -amidating monooxygenase (PAM) — activates neuropeptides (oxytocin, vasopressin, CRH, CCK)
- Hypoxia-inducible factor (HIF) regulation: PHD enzymes (prolyl hydroxylases) regulate HIF-1 α degradation; ascorbate is a cofactor; implications for tumor angiogenesis and O_2 sensing
- Epigenetics: TET dioxygenases (DNA demethylation via 5mC $\rightarrow$ 5hmC) require ascorbate — emerging role in cancer biology and stem cell pluripotency

V. KEY CLINICAL SYNDROMES — QUICK REFERENCE

Deficiency / Syndrome	Vitamin(s) Key Clinical Features
Wernicke-Korsakoff	B1 (Thiamin) Ophthalmoplegia, ataxia, amnesia; alcoholism, refeeding risk
Pellagra (4 Ds)	B3 (Niacin) Dermatitis, Diarrhea, Dementia, Death
Megaloblastic anemia	Folate + B12 Macrocytic anemia (distinguish by neuro findings for B12)
Subacute combined degeneration	B12 Dorsal/lateral column demyelination; spastic ataxia
Neural tube defects	Folate Anencephaly, spina bifida; periconceptional supplementation prevents
Homocysteinemia	B12, Folate, B6 $\uparrow$ CVD, VTE, cognitive decline risk; MTHFR polymorphism
Scurvy	Vitamin C Bleeding gums, perifollicular hemorrhage, poor wound healing
Sideroblastic anemia	B6 (PLP) Ringed sideroblasts; $\downarrow$ heme synthesis (ALA synthase)
Ariboflavinosis	B2 Glossitis, cheilosis, seborrheic dermatitis; impairs other B vitamins
Peripheral neuropathy (megadose)	B6 >200 mg/day chronic — sensory neuropathy without motor involvement

Lecture 2 Summary — Key Principles

- B vitamins function as coenzyme precursors — each has specific metabolic niches (energy metabolism, 1C metabolism, neurotransmitter synthesis, heme synthesis)
- One-carbon metabolism requires the orchestrated action of folate, B12, B6, and riboflavin — deficiency in any disrupts the entire network, elevating homocysteine
- B12 and folate deficiency share megaloblastic anemia but diverge in neurological manifestations — distinguishing them is a critical clinical skill
- Vitamin C is not just an antioxidant — it is a required cofactor in collagen synthesis, carnitine production, neurotransmitter synthesis, and iron absorption
- Gateway deficiencies: riboflavin deficiency impairs activation of B6, folate, and niacin; B12 deficiency causes functional folate deficiency (methyl trap)

Suggested Reading:

1. Stipanuk MH & Caudill MA (2019). Biochemical, Physiological & Molecular Aspects of Human Nutrition, 4th Ed. |
2. Bailey LB (ed). Folate in Health and Disease, 2nd Ed. |
3. Institute of Medicine DRI Reports.

LECTURE 3

Minerals & Phytonutrients

Major & Trace Minerals | Phytonutrients & Antioxidant Protective Elements

Nutrition & Dietetics MS Program | Department of Nutritional Sciences

Learning Outcomes — By the end of this lecture, students will be able to:

- Describe the absorption mechanisms and regulation of major minerals (Ca, Mg, P) and key trace elements (Fe, Zn, I, Se, Cu, Cr)
- Explain mineral-mineral interactions and their clinical implications (Fe/Zn/Ca competition, heme vs. non-heme iron)
- Classify phytonutrients by chemical class (polyphenols, carotenoids, glucosinolates, organosulfur) and describe mechanisms of protective action
- Evaluate the epidemiological and clinical evidence for phytonutrient roles in chronic disease prevention
- Apply knowledge of bioavailability-modifying factors in dietary assessment and counseling

I. MINERALS — FOUNDATIONAL CONCEPTS

A. Classification

- Major (macro) minerals: Require >100 mg/day; Ca, P, Mg, Na, K, Cl, S
- Trace minerals (microminerals): Require <100 mg/day; Fe, Zn, I, Se, Cu, Mn, Cr, Mo, F, Co
- Ultra-trace minerals: Require microgram quantities; Si, Ni, V, B (boron), Sn — essentiality variable/debated

B. Bioavailability Modifiers — Key for Clinical Practice

Factor	Effect on Mineral Absorption
Phytate (inositol hexaphosphate)	Chelates Fe, Zn, Ca, Mg → ↓ absorption; reduced by soaking, fermentation, sprouting, phytase
Oxalate	Precipitates Ca and Mg → ↓ absorption; spinach Ca has <5% bioavailability
Tannins (polyphenols)	Bind Fe ³⁺ and Zn ²⁺ → inhibit absorption; in tea, coffee, red wine
Vitamin C	Reduces Fe ³⁺ → Fe ²⁺ → greatly enhances non-heme iron absorption
MFP factor (meat-fish-poultry)	Enhances non-heme iron absorption; mechanism incompletely understood
Calcium	Competes with Zn, Fe, Mg absorption at high doses → separate supplementation timing
Vitamin D (calcitriol)	Upregulates Ca absorption proteins (TRPV6, calbindin) → ↑ Ca absorption
Body iron status	↑ Transferrin receptor expression → ↑ iron absorption when stores low (regulated by hepcidin)

II. MAJOR MINERALS

A. Calcium

Most abundant mineral in the body (~1000 g in 70 kg adult); 99% in skeleton (hydroxyapatite: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) and 1% in blood/soft tissues (extracellular Ca^{2+} = ~2.5 mmol/L; intracellular Ca^{2+} = ~100 nmol/L — 25,000-fold gradient).

Absorption:

- Active transcellular (saturable, vitamin D-dependent): Primarily in duodenum/proximal jejunum; involves TRPV6 (apical Ca^{2+} entry), calbindin-D9k (cytoplasmic ferry), PMCA1b (basolateral extrusion); occurs at low-moderate intake; efficiency 30–40% but regulated
- Passive paracellular (diffusion-dependent): Throughout small intestine; proportional to luminal Ca^{2+} concentration; predominant at high Ca^{2+} intakes (supplements); efficiency ~10%

Homeostasis — Ca^{2+} Triad:

- PTH (parathyroid hormone): $\uparrow$ when $\text{Ca}^{2+} \downarrow \rightarrow \uparrow$ bone resorption ($\uparrow$ osteoclasts via RANKL), $\uparrow$ renal Ca^{2+} reabsorption (DCT), $\uparrow$ calcitriol synthesis $\rightarrow$ net $\uparrow$ plasma Ca^{2+}
- Calcitriol ($1,25(\text{OH})_2 \text{D}$): $\uparrow$ intestinal Ca^{2+} absorption; amplifies PTH effects on bone; $\uparrow$ renal reabsorption
- Calcitonin (thyroid C cells): $\uparrow$ when $\text{Ca}^{2+} \uparrow \rightarrow \downarrow$ osteoclast activity $\rightarrow \downarrow$ bone resorption; pharmacological use in hypercalcemia/Paget's disease

Functions beyond bone:

- Second messenger: Intracellular Ca^{2+} transients $\rightarrow$ signal transduction (calmodulin, CaM kinases, PKC, calpains)
- Neuromuscular function: Action potential repolarization, neurotransmitter release (SNARE complex), muscle contraction (troponin C)
- Blood coagulation: Multiple clotting factors are Ca^{2+} -dependent

Clinical Note: Osteoporosis: Peak bone mass achieved ~25–30 years; influenced by lifetime Ca + vitamin D intake, physical activity, estrogen/testosterone, genetics. Fracture risk is the key outcome. Dietary Ca from food sources preferred over supplements; supplement Ca (CaCO_3) associated with $\uparrow$ cardiovascular events in some meta-analyses (not observed with dietary Ca). RDA: 1,000–1,200 mg/day depending on age/sex.

B. Magnesium

Fourth most abundant cation in the body; ~60% in bone, ~39% intracellular (muscle, liver), ~1% in blood. Intracellular Mg^{2+} is the second most abundant cation after K^+ . Sources: nuts, seeds, legumes, whole grains, leafy greens, dark chocolate. RDA: 310–420 mg/day.

Critical biochemical roles:

- Cofactor for >300 enzymes: Including all ATP-utilizing enzymes (ATP exists in cells as Mg-ATP complex), and all kinases, synthases, phosphatases
- Nucleotide metabolism: Required for DNA and RNA polymerases, helicases, topoisomerases
- NMDA receptor: Mg^{2+} blocks the receptor channel at rest; depolarization relieves Mg^{2+} block $\rightarrow \text{Ca}^{2+}$ influx $\rightarrow$ long-term potentiation (memory/learning)
- Cardiovascular: Regulates vascular tone, inhibits platelet aggregation, stabilizes cardiac membrane potential

Clinical Note: Hypomagnesemia (common in ICU, alcohol use disorder, malabsorption, diuretic use, poorly controlled T2DM, PPIs): Can cause hypocalcemia ($\downarrow$ PTH secretion and PTH resistance) and hypokalemia ($\downarrow$ K^+ renal conservation). Look for refractory hypokalemia that doesn't correct without Mg replacement. Neurological signs: tetany, seizures, personality changes.

C. Phosphorus

Second most abundant mineral; ~85% in bone (hydroxyapatite), ~14% intracellular (organic phosphate in ATP, phospholipids, proteins), ~1% extracellular. Absorption is highly efficient (~65–70% of dietary intake); regulated by FGF-23 (phosphatonin) and calcitriol. Sources: widespread in protein-containing foods; phosphate additives in processed foods significantly increase intake. RDA: 700 mg/day (adults).

Key functions:

- Energy currency: ATP, ADP, AMP — creatine phosphate in muscle
- Cell signaling: cAMP, cGMP, protein phosphorylation/dephosphorylation (kinase/phosphatase cascades)
- Structural: Phospholipid bilayers (cell membranes), nuclear membrane, myelin
- Oxygen delivery: 2,3-DPG in RBCs regulates hemoglobin- O_2 affinity
- Acid-base buffer: $H_2PO_4^- / HPO_4^{2-}$ system (pKa = 6.8; important in urine)

FGF-23 and Phosphate: FGF-23 (from osteocytes) $\rightarrow$ $\downarrow$ renal 1α -hydroxylase $\rightarrow$ $\downarrow$ calcitriol $\rightarrow$ $\downarrow$ intestinal P absorption + $\uparrow$ renal P excretion. Key in CKD-MBD (chronic kidney disease-mineral bone disorder).

III. TRACE MINERALS

A. Iron — Most Studied Trace Mineral

Total body iron ~3.5–4 g (70 kg adult): ~65% hemoglobin, ~10% myoglobin/enzymes, ~25% stored as ferritin/hemosiderin (liver, spleen, bone marrow). Daily losses: ~1–2 mg (sweat, GI, skin cell turnover); menstruating women: additional 1–2 mg/day average.

Absorption — Heme vs. Non-Heme:

- Heme iron (Fe-protoporphyrin): From meat/fish/poultry hemoglobin and myoglobin; absorbed intact via HCP1 transporter; bioavailability 15–35%; NOT inhibited by phytate, tannins, or calcium
- Non-heme iron (ionic): Majority of dietary iron; Fe^{3+} reduced to Fe^{2+} by duodenal cytochrome b (Dcytb, requires ascorbate) $\rightarrow$ transported by DMT-1 (divalent metal transporter 1); bioavailability 2–20%; highly affected by dietary inhibitors/enhancers

Iron regulatory proteins (IRP) — cellular iron sensing:

- When iron is low: IRPs bind Iron Response Elements (IREs) on mRNA $\rightarrow$ stabilize transferrin receptor (TfR) mRNA $\rightarrow$ $\uparrow$ TfR expression ($\uparrow$ uptake) AND suppress ferritin mRNA translation ($\downarrow$ storage)
- When iron is high: IRPs inactive $\rightarrow$ $\downarrow$ TfR expression, $\uparrow$ ferritin synthesis (sequester excess)

Hepcidin — systemic iron regulation:

Hepcidin (liver-derived antimicrobial peptide) is the master regulator of systemic iron homeostasis. $\uparrow$ Hepcidin $\rightarrow$ binds and degrades ferroportin (iron exporter on enterocytes, macrophages, hepatocytes) $\rightarrow$ blocks iron absorption and release from stores. Stimulated by: iron loading, inflammation (IL-6), BMP signaling. Suppressed by: iron deficiency, hypoxia, erythropoiesis, HIF-2 α .

Clinical Note: *Anemia of inflammation/chronic disease: $\uparrow$ IL-6 $\rightarrow$ $\uparrow$ hepcidin $\rightarrow$ $\uparrow$ ferroportin degradation $\rightarrow$ functional iron deficiency (iron trapped in macrophages) despite normal/elevated stores. Serum ferritin is elevated (positive acute phase reactant) — cannot assess iron stores normally. Soluble TfR and TfR:ferritin ratio may help distinguish. IV iron and ESAs used therapeutically.*

Iron deficiency stages:

- Stage 1 (Iron depletion): $\downarrow$ ferritin; no anemia; serum Fe and Hgb normal
- Stage 2 (Iron-deficient erythropoiesis): $\downarrow$ ferritin, $\downarrow$ serum Fe, $\uparrow$ TIBC, $\uparrow$ TfR; no anemia yet
- Stage 3 (Iron deficiency anemia, IDA): Microcytic, hypochromic anemia; $\downarrow$ Hgb, $\downarrow$ MCV, $\downarrow$ MCH; classic fatigue, pallor, pica, restless legs

B. Zinc

~2–3 g total body Zn; no specific storage depot (unlike iron) — deficiency develops relatively rapidly. Sources: oysters (highest), red meat, poultry, seafood, whole grains, legumes, nuts. RDA: 8 mg/day (women), 11 mg/day (men).

Biochemical roles:

- Catalytic: Active site of >300 enzymes — carbonic anhydrase, carboxypeptidase, alcohol dehydrogenase, alkaline phosphatase, Cu/Zn-SOD, DNA polymerase, RNA polymerase
- Structural: Zinc finger proteins — transcription factors (steroid hormone receptors, p53, SP1) — Zn^{2+} coordinates cysteine/histidine residues to stabilize DNA-binding domain
- Regulatory: PKC activation; redox regulation (Zn is redox-inert itself, but regulates thiol groups on proteins)

Clinical Note: *Deficiency manifestations: Growth retardation, hypogonadism, delayed sexual maturation, acrodermatitis enteropathica-like rash (perioral, perianal, acral), poor wound healing, alopecia, impaired taste (hypogeusia), immune dysfunction. Acrodermatitis enteropathica: autosomal recessive mutation in Zip4 (zinc transporter SLC39A4) $\rightarrow$ severe deficiency in infancy. Plasma Zn is an insensitive marker; no ideal biomarker — functional assessment (taste acuity, taste-response test) used adjunctively.*

C. Iodine & Thyroid Axis

Iodine is primarily concentrated in the thyroid gland (~70–80% of body iodine). Sources: iodized salt, seafood, dairy (iodophor sanitizers), seaweed. RDA: 150 $\mu\text{g}/\text{day}$; pregnancy 220 $\mu\text{g}/\text{day}$. Global iodine deficiency disorders (IDD) are the leading preventable cause of intellectual disability worldwide.

Thyroid hormone synthesis: I^- transported into thyroid follicular cells by Na/I symporter (NIS) $\rightarrow$ oxidized by thyroid peroxidase (TPO) in presence of H_2O_2 $\rightarrow$ iodination of tyrosine residues on thyroglobulin $\rightarrow$ MIT, DIT $\rightarrow$ coupling $\rightarrow$ T3 (triiodothyronine) and T4 (thyroxine) $\rightarrow$ stored in follicular colloid $\rightarrow$ released upon TSH stimulation $\rightarrow$ T4 peripherally deiodinized to active T3 (by deiodinases, selenium-dependent).

Clinical Note: *Goiter: $\downarrow$ thyroid hormone $\rightarrow$ $\uparrow$ TSH $\rightarrow$ thyroid gland hypertrophy. Cretinism (congenital hypothyroidism): Maternal iodine deficiency during pregnancy $\rightarrow$ severe intellectual disability, deaf-mutism, motor deficits; prevented by universal salt iodization. Iodine excess (Wolff-Chaikoff effect): Acute excess transiently inhibits thyroid hormone synthesis — usually escaped after adaptation, but can cause hypothyroidism in susceptible individuals.*

Goitrogens: compounds interfering with iodine utilization:

- Thiocyanates: Competitive inhibitors of NIS; from glucosinolates in cruciferous vegetables (cassava, cabbage, broccoli) → significant only with very high intake + marginal iodine status
- Isoflavones (soy): May inhibit TPO at high doses — evidence mixed; concern mainly in iodine-deficient populations

D. Selenium

Selenium is incorporated as selenocysteine (the 21st amino acid) into selenoproteins via UGA codon recoding. ~25 human selenoproteins identified. Sources: Brazil nuts (highly variable; 1–2 nuts can meet daily needs), seafood, meat, whole grains. RDA: 55 µg/day. UL = 400 µg/day (selenosis risk).

Key selenoproteins:

- Glutathione peroxidases (GPx1–6): Reduce H_2O_2 and lipid hydroperoxides using glutathione — primary selenium-dependent antioxidant enzymes; coordinate with vitamin E in antioxidant defense
- Thioredoxin reductases (TrxR1–3): Regenerate thioredoxin (Trx) → reduces oxidized proteins, ribonucleotide reductase (DNA synthesis), thioredoxin-peroxidases; also regenerates ascorbate and vitamin E
- Iodothyronine deiodinases (DIO1–3): Activate T4 → T3 (DIO1, 2) and inactivate thyroid hormones (DIO3) — Se deficiency can impair thyroid function
- Selenoprotein P (SELENOP): Primary selenium transport protein in plasma; marker of selenium status; antioxidant role in vasculature

Clinical Note: *Keshan disease: Selenium-deficient cardiomyopathy (endemic in Keshan region of China); prevented by selenium supplementation. Kashin-Beck disease: Osteoarthropathy in Se-deficient regions. Note: ATBC and SELECT trials failed to demonstrate cancer prevention by Se supplementation in replete populations — context of baseline status matters critically.*

E. Copper

~75–150 mg total body Cu; concentrated in liver, brain, heart, kidneys. Sources: organ meats (liver, highest), shellfish (oysters), nuts, seeds, legumes. RDA: 900 µg/day. Absorbed via CTR1 transporter; exported from enterocytes and liver via ATP7A and ATP7B (ATPases).

Key copper metalloenzymes:

- Cu/Zn-SOD (SOD1): Cytosolic dismutation of superoxide → H_2O_2 ; antioxidant defense
- Cytochrome c oxidase (Complex IV): ETC — Cu and heme; ~80% mitochondrial energy production
- Lysyl oxidase: Cross-links collagen and elastin → structural integrity of connective tissue; deficiency → Menkes kinky hair syndrome (vascular fragility, aortic aneurysm, bone fractures)
- Ceruloplasmin: Plasma Cu transport; ferroxidase activity ($Fe^{2+} \rightarrow Fe^{3+} \rightarrow$ loads transferrin); ↓ in Wilson disease
- Dopamine β-hydroxylase: Dopamine → norepinephrine (adrenergic function)

Clinical Note: *Wilson's disease (AR): Mutation in ATP7B → Cu accumulates in liver, brain, cornea (Kayser-Fleischer rings), kidneys. Menkes disease (X-linked): Mutation in ATP7A → Cu cannot exit enterocytes → severe deficiency in all tissues. Supplemental zinc (>50 mg/day) induces metallothionein in enterocytes → traps Cu → useful clinically to reduce Cu absorption in Wilson's disease.*

IV. PHYTONUTRIENTS & PROTECTIVE ELEMENTS

A. Definitions & Classification

Phytonutrients (phytochemicals) are non-essential bioactive plant compounds that are not traditional vitamins or minerals but have demonstrable health-promoting effects. Estimated >25,000 phytochemicals in plant foods; most studied are polyphenols, carotenoids, glucosinolates, and organosulfur compounds.

B. Polyphenols — The Largest Class

Polyphenols share a basic structure of one or more aromatic rings with hydroxyl groups. Highly diverse: >8,000 structures identified. Generally poor and variable bioavailability (extensive phase II metabolism; colonic microbiome-dependent transformation to active metabolites).

Polyphenol Subclass	Key Representatives & Sources
Flavonols	Quercetin, kaempferol, myricetin — onions, kale, broccoli, apples, berries, tea
Flavones	Apigenin, luteolin — parsley, celery, chamomile tea
Flavanones	Hesperidin, naringenin — citrus fruits, especially the peel/pith
Flavan-3-ols (catechins)	Epicatechin, EGCG — green tea (richest EGCG source), cocoa/dark chocolate, grapes
Anthocyanins	Cyanidin, delphinidin, malvidin — berries, red/purple grapes, red cabbage, eggplant
Isoflavones	Genistein, daidzein — soybeans, edamame, tempeh; phytoestrogenic
Stilbenes	Resveratrol — red grape skins, red wine, peanuts; SIRT1 activation
Phenolic acids	Chlorogenic acid, caffeic acid — coffee (chlorogenic acid, largest single polyphenol in Western diet), berries
Lignans	Secoisolariciresinol — flaxseeds (richest source), whole grains; enterolactone/enterodiol via gut bacteria
Tannins	Ellagitannins, proanthocyanidins — pomegranate, walnuts, berries, grape seed

Mechanisms of Polyphenol Action:

- Antioxidant: Direct ROS scavenging; metal chelation (Fe, Cu) — prevent Fenton chemistry; Nrf2 pathway activation (↑ endogenous antioxidant enzymes: HO-1, SOD, CAT, GPx, GCL)
- Anti-inflammatory: Inhibit NF-κB pathway → ↓ COX-2, ↓ cytokines (TNF-α, IL-1β, IL-6); inhibit arachidonic acid release (↓ PLA₂)
- Enzyme modulation: Inhibit Phase I enzymes (CYP1A1, CYP1B1) that activate procarcinogens; induce Phase II detoxification enzymes (GST, UGT) — increases carcinogen elimination
- Cell signaling: EGFR inhibition; SIRT1 activation (resveratrol); AMPK activation; mTOR inhibition — anti-proliferative/autophagy induction
- Gut microbiome modulation: Many polyphenols reach the colon intact → prebiotic effect; fermented to short-chain fatty acids (SCFAs) and bioactive metabolites (urolithins from ellagitannins; equol from isoflavones)
- Epigenetic: HDAC inhibition (butyrate from polyphenol fermentation); DNA methylation modulation; miRNA regulation

C. Non-Provitamin A Carotenoids

Carotenoids are C40 isoprenoid pigments. >600 identified in nature; ~20 in human blood; primarily from dark orange/red/yellow and dark green plant foods. Fat-soluble; absorption enhanced by co-ingestion of fat and heating/processing (lycopene in cooked tomatoes 2–4x more bioavailable than raw).

Carotenoid	Primary Sources & Proposed Health Roles
Lycopene	Tomatoes (esp. cooked, concentrated products), watermelon, pink grapefruit — epidemiological association with ↓ prostate cancer, CVD; potent singlet O ₂ quencher
Lutein & Zeaxanthin	Kale, spinach, corn, eggs, orange peppers — concentrated in retinal macula; reduce oxidative damage from light; associated with ↓ AMD and cataract risk; brain lutein correlates with cognitive performance
Astaxanthin	Salmon, shrimp, krill — most potent carotenoid antioxidant; crosses BBB; anti-inflammatory; sports performance research
β-Cryptoxanthin	Oranges, papayas, tangerines — provitamin A + antioxidant; associated with ↓ lung cancer, ↓ arthritis risk

Clinical Note: *AREDS (Age-Related Eye Disease Study) and AREDS2: Lutein/Zeaxanthin supplementation (10mg/2mg) reduced progression to advanced AMD by ~25%; replaced β-carotene in AREDS2 formula (β-carotene ↑ lung cancer risk in smokers). One of the strongest evidence-based applications of phytonutrient supplementation.*

D. Glucosinolates & Isothiocyanates (Cruciferous Vegetables)

Glucosinolates are sulfur-containing glycosides found in all cruciferous vegetables (Brassica family): broccoli, Brussels sprouts, cabbage, kale, cauliflower, bok choy, arugula, radish, watercress. When cells are damaged (chopping, chewing) → myrosinase enzyme hydrolyzes glucosinolates → isothiocyanates (ITCs), nitriles, indoles.

Key isothiocyanates and indoles:

- Sulforaphane (from glucoraphanin, abundant in broccoli/broccoli sprouts): Most studied ITC. Potent activator of Nrf2 transcription factor → upregulates Phase II detoxification enzymes (GST, NQO1, HO-1) and antioxidant proteins → cancer chemopreventive activity (Phase 1/2 clinical trials in prostate, breast, lung, colon). Also inhibits HDAC and NF-κB; anti-inflammatory in preclinical models. Broccoli sprouts have 10–100x higher glucoraphanin than mature broccoli heads.
- Indole-3-carbinol (I3C) and DIM (diindolylmethane): From indole glucosinolates; modulate estrogen metabolism (favor 2-OH vs. 16α-OH estrogen metabolites) → potential protective against hormone-dependent cancers; weak ER antagonists
- Phenethyl isothiocyanate (PEITC): From watercress; inhibits tobacco carcinogen activation (CYP2E1); Phase II data in lung cancer prevention in smokers

Bioavailability note: Myrosinase is heat-labile → boiling cruciferous vegetables significantly reduces ITC formation; steaming, microwaving, and raw consumption preferred. Gut microbiota also express myrosinase activity — important when vegetables are cooked.

E. Organosulfur Compounds (Allium Vegetables)

Allium vegetables (garlic, onions, leeks, chives, shallots, scallions) contain unique organosulfur compounds formed from S-alk(en)yl-cysteine sulfoxides via alliinase enzyme (activated by crushing/chopping):

- Allicin (diallyl thiosulfinate): Primary bioactive in fresh garlic; unstable → rapidly converts to diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoene, vinylthiins
- S-Allylcysteine (SAC): Water-stable; present in aged black garlic; most bioavailable form; AMPK activation; antioxidant

Demonstrated mechanisms:

- Cardiovascular: Modest but significant ↓ LDL-C, ↓ total cholesterol, ↓ blood pressure (systematic review/meta-analysis support); inhibit platelet aggregation (↓ TXA₂); ↑ fibrinolysis
- Antimicrobial: Broad-spectrum activity against H. pylori, S. aureus, Candida — allicin disrupts thiol-dependent microbial enzymes
- Anti-carcinogenic: Inhibit CYP2E1 (carcinogen activation); ↑ GSH; induce apoptosis (DATS) in cancer cell lines
- Immune modulation: ↑ NK cell activity; ↑ macrophage phagocytosis; ↑ T-cell proliferation

F. Other Notable Protective Phytonutrients

Curcuminoids (Turmeric):

Curcumin: Primary polyphenol in Curcuma longa (turmeric). Potent NF-κB inhibitor, Nrf2 activator, HDAC inhibitor, and epigenetic regulator. Extensively studied in over 100 clinical trials. Major limitation: extremely poor bioavailability (rapid metabolism, low solubility). Bio-enhancement strategies: piperine (BioPerine®), nanoparticles, liposomal formulations, phospholipid complexes.

Resveratrol (Stilbene):

Trans-resveratrol: SIRT1 activator → NAD⁺-dependent deacetylation of PGC-1α, FOXO, p53 → metabolic effects mimicking caloric restriction. Also direct AMPK activator; anti-angiogenic; inhibits platelet aggregation. Limited clinical evidence for cardiovascular outcomes despite promising mechanistic data — attributed largely to poor bioavailability.

Omega-3 fatty acids (as protective elements in diet):

- EPA and DHA (marine omega-3s): Incorporated into cell membranes → alter eicosanoid profile (↓ pro-inflammatory PGE₂, TXA₂ from AA; ↑ resolvins, protectins, maresins from EPA/DHA — specialized pro-resolving mediators)
- Strong evidence: ↓ triglycerides (4 g/day EPA/DHA: ~30–45% ↓ TG); ↓ fatal cardiac events; ↓ inflammation (CRP, IL-6); neuroprotective
- Recent trials: REDUCE-IT (icosapentaenoic acid 4 g/day) → significant ↓ major cardiovascular events beyond statin therapy (HR 0.75)

V. BIOAVAILABILITY, FOOD MATRIX & DIETARY PATTERNS

A critical principle for MS-level dietetics: isolated phytonutrients in supplements rarely replicate the effects of whole food consumption. Reasons include:

- Food matrix effects: Phytonutrients in whole foods exist in a complex matrix with fiber, lipids, and other phytochemicals that modulate absorption, metabolism, and bioavailability
- Synergistic interactions: Combinations of phytonutrients (polyphenols + carotenoids + vitamins + minerals) exert synergistic effects not achievable by isolated compounds — the antioxidant network
- Microbiome-dependent activation: Many phytonutrients (ellagitannins → urolithins; isoflavones → equol; lignans → enterolactone) require gut microbial transformation to their active forms — individual response highly dependent on microbiome composition
- Hormesis: Many phytonutrients act via hormetic mechanisms — low doses activate protective pathways (Nrf2, SIRT1, AMPK); high supplemental doses may be ineffective or harmful

The Mediterranean diet, DASH diet, and plant-forward dietary patterns — rich in diverse polyphenols, carotenoids, glucosinolates, fiber, and phytosterols — consistently show strongest evidence for chronic disease prevention in large-scale cohort studies and some RCTs (PREDIMED trial: Mediterranean diet + olive oil or nuts → 30% ↓ major cardiovascular events).

Lecture 3 Summary — Key Principles

- Mineral bioavailability is as important as intake — phytate, oxalate, tannins, and mineral-mineral competition are critical factors for dietary assessment
- Iron regulation (hepcidin/ferroportin axis, IRP/IRE system) is tightly controlled at multiple levels; anemia of chronic disease differs mechanistically from IDA
- Selenium-dependent selenoproteins (GPx, TrxR, deiodinases) coordinate antioxidant defense, thyroid hormone activation, and DNA synthesis
- Phytonutrients are not nutrients but bioactive compounds; classification: polyphenols (flavonoids, phenolic acids, stilbenes), carotenoids, glucosinolates/ITCs, organosulfur
- Sulforaphane (broccoli), lycopene, lutein/zeaxanthin, allicin, and curcumin are among the most evidence-based phytonutrients for chronic disease prevention
- Whole food > isolated supplement: bioavailability, food matrix effects, microbiome activation, and synergistic interactions favor dietary patterns over phytonutrient supplements

Suggested Reading:

1. Goff JP (2018). Minerals. In: Stipanuk MH & Caudill MA (Eds.), Biochemical, Physiological & Molecular Aspects of Human Nutrition, 4th Ed. |
2. Manach C et al. (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr. |
3. Fahey JW et al. Sulforaphane: from bench to bedside. Annu Rev Nutr. |
4. PREDIMED trial publications (NEJM 2013, 2018 correction).

Module 2

Body Regulation & Composition

Professor Osama Awad Salih

NUTRITION & DIETETICS

MS Program Lecture Series

Body Regulation & Composition

Internal Balance & Physical Assessment

Fluids & Electrolytes • Body Composition Across the Lifespan

Professor Osama Awad Salih

Lecture 1

Fluids & Electrolytes

Body fluids • Electrolyte regulation
pH balance • Clinical disorders

Lecture 2

Body Composition

Compartment models • Lifespan changes
Measurement techniques • Clinical tools

LECTURE 1

Fluids & Electrolytes

Body Fluid Compartments, Electrolyte Physiology, pH Balance & Clinical Disorders

Nutrition & Dietetics MS Program | Body Regulation & Composition Series

Learning Outcomes — By the end of this lecture, students will be able to:

- Map the body fluid compartments and quantify fluid distribution across total body water, intracellular, and extracellular spaces
- Describe the mechanisms governing water movement: osmosis, oncotic pressure, Starling forces, and hydrostatic gradients
- Explain the homeostatic regulation of sodium, potassium, chloride, calcium, magnesium, and phosphate
- Trace the three-buffer systems for pH regulation: bicarbonate/carbonic acid, phosphate, and protein buffers
- Identify primary acid-base disorders (metabolic and respiratory acidosis/alkalosis) and apply the compensation framework
- Diagnose electrolyte disorders from clinical and laboratory data and apply evidence-based dietary interventions
- Apply fluid and electrolyte knowledge to clinical nutrition scenarios: refeeding syndrome, dehydration, CKD, and exercise

I. BODY FLUID COMPARTMENTS & DISTRIBUTION

A. Total Body Water (TBW) — Overview

Water is the most abundant molecule in the human body and the medium for all biochemical reactions. Total body water (TBW) varies substantially with age, sex, and body composition — primarily because adipose tissue is relatively anhydrous (~10% water) while skeletal muscle is ~75% water.

Population Group	TBW as % Body Weight Approximate Volume (70 kg)
Adult male (lean)	~60% ~42 L
Adult female (lean)	~50–55% ~35–38 L
Obese adults (both sexes)	~45–50% (lower % due to high fat mass)
Neonates / infants	~75–80% (high TBW; low fat)
Elderly (>65 years)	~45–50% (↓ muscle mass → ↓ TBW; ↑ dehydration risk)

B. Fluid Compartments & Composition

TBW is divided into two primary compartments separated by the cell membrane:

- Intracellular Fluid (ICF): ~60% of TBW (~25 L in 70 kg adult). Dominant cation: K^+ (~140 mEq/L); dominant anions: organic phosphates (ATP, glucose-6-P), proteins. High K^+ maintained by Na^+/K^+ -ATPase (3 Na^+ out : 2 K^+ in per cycle, using ATP)
- Extracellular Fluid (ECF): ~40% of TBW (~17 L). Dominant cation: Na^+ (~140 mEq/L); dominant anions: Cl^- (~104 mEq/L) and HCO_3^- (~24 mEq/L). ECF is subdivided into:
 - Plasma (intravascular): ~3 L; contains plasma proteins (albumin, globulins) → generates oncotic pressure
 - Interstitial fluid: ~12 L; surrounds cells; protein-poor; mediates exchange between plasma and cells
 - Transcellular fluids: ~1–2 L; specialized secretions — CSF, synovial, pleural, peritoneal, GI, intraocular fluids; clinically significant in disease states (ascites, pleural effusion)

Property	ICF (~25 L)	ECF (~17 L)
Dominant cation	K^+ (140 mEq/L)	Na^+ (140 mEq/L)
Dominant anion	Phosphate, proteins, HCO_3^-	Cl^- (104), HCO_3^- (24 mEq/L)
Osmolality	~285 mOsm/kg	~285–295 mOsm/kg
Mg^{2+}	~20 mEq/L (active metabolic role)	~1–2 mEq/L (serum)
Ca^{2+}	<0.0001 mEq/L (tightly controlled signalling)	~2.4 mmol/L total (ionised ~1.1–1.3 mmol/L)
Proteins	High (enzymes, structural)	Low — only in plasma (~7 g/dL albumin + globulins)

C. Forces Governing Water Movement

1. Osmosis & Osmolality

Water moves passively across semi-permeable membranes from areas of low solute concentration to high solute concentration (osmosis) via aquaporin water channels (AQP1–4 in key tissues). Osmolality = solute concentration per kg water (mOsm/kg H_2O).

Serum Osmolality Estimation (Calculated):

$$P_{osm} = 2[Na^+] + [Glucose]/18 + [BUN]/2.8$$

Normal range: 285–295 mOsm/kg. Na^+ dominates (~90% of ECF osmolality). Osmolal gap = Measured – Calculated (>10 mOsm/kg suggests unmeasured osmoles: ethanol, methanol, ethylene glycol, mannitol)

2. Starling Forces — Capillary Fluid Exchange

Fluid movement between plasma and interstitium is governed by Starling forces — the balance of hydrostatic and oncotic pressures across the capillary wall:

Starling Equation (Net Filtration Pressure):

$$NFP = [(P_c - P_i) - \sigma(\pi_c - \pi_i)] \times K_f$$

P_c = capillary hydrostatic; P_i = interstitial hydrostatic; π_c = plasma oncotic (~25 mmHg, from albumin); π_i = interstitial oncotic; σ = reflection coefficient; K_f = filtration coefficient

- At arteriolar end: P_c high (~ 35 mmHg) $>$ π_c (~ 25 mmHg) $\rightarrow$ net filtration OUT of capillary $\rightarrow$ fluid enters interstitium
- At venular end: P_c falls (~ 15 mmHg) $<$ π_c (~ 25 mmHg) $\rightarrow$ net reabsorption $\rightarrow$ fluid returns to capillary
- Residual filtered fluid ($\sim 2\text{--}4$ L/day) $\rightarrow$ lymphatic drainage $\rightarrow$ returned to venous circulation

Clinical Note: *Oedema mechanisms: (1) $\downarrow$ plasma oncotic pressure (hypoalbuminaemia from malnutrition, cirrhosis, nephrotic syndrome); (2) $\uparrow$ capillary hydrostatic pressure (heart failure, venous obstruction); (3) $\uparrow$ capillary permeability (inflammation, sepsis); (4) lymphatic obstruction. Differentiating the mechanism guides nutrition intervention — albumin repletion is appropriate only if synthesis is limiting.*

3. Tonicity vs. Osmolality

Tonicity (effective osmolality) describes only the solutes that cannot freely cross cell membranes — i.e., solutes that actually drive water movement. Urea crosses freely $\rightarrow$ does not affect tonicity (ineffective osmole). Na^+ and glucose are effective osmoles. Clinically: hypertonic saline (3% NaCl) causes water to move OUT of cells; hypotonic IV solutions (0.45% NaCl) move water INTO cells.

II. WATER HOMEOSTASIS — INTAKE, REGULATION & LOSSES

A. Water Intake & Requirements

Daily water intake comes from beverages ($\sim 1.2\text{--}1.5$ L), food water ($\sim 0.7\text{--}1.0$ L), and metabolic water from oxidation of macronutrients ($\sim 0.25\text{--}0.35$ L). The Adequate Intake (AI) for total water is 3.7 L/day (men) and 2.7 L/day (women), encompassing all beverage and food sources — not simply drinking water.

Route of Water Loss	Approximate Daily Volume Key Notes
Urine	1,000–2,000 mL; obligatory minimum ~ 500 mL/day (to excrete solute load); regulated by ADH
Insensible losses — Skin	350–400 mL; unavoidable diffusion through epidermis (not sweat); $\uparrow$ with fever, burns, dry environments
Insensible losses — Respiratory	250–350 mL; exhaled water vapour; $\uparrow$ with hyperventilation, high altitude, exercise
Sweat (sensible)	100–8,000+ mL; highly variable; contains Na^+ (20–80 mEq/L), Cl^- , K^+ , Mg^{2+} ; trained athletes sweat more volume but lower Na^+ concentration
Faecal	100–200 mL; $\uparrow\uparrow$ in diarrhoea (cholera: up to 20 L/day); large intestine normally reabsorbs $\sim 1,400$ mL/day

B. Antidiuretic Hormone (ADH/AVP) — The Primary Water Regulator

Arginine vasopressin (AVP/ADH), synthesized in hypothalamic paraventricular and supraoptic nuclei, is the central hormone governing renal water reabsorption.

- Stimuli for ADH release: ↑ plasma osmolality (sensed by osmoreceptors in OVLT and SFO, threshold ~280–285 mOsm/kg); ↓ blood volume/pressure (sensed by carotid sinus and aortic arch baroreceptors — volume overrides osmolality in severe depletion); nausea, pain, stress (non-osmotic stimuli)
- Mechanism: ADH binds V2 receptors on principal cells of renal collecting duct → cAMP/PKA → inserts aquaporin-2 (AQP2) vesicles into apical membrane → water reabsorption along osmotic gradient into hypertonic medullary interstitium
- Result: More concentrated urine (up to 1,200 mOsm/kg), ↓ urine volume, ↑ plasma volume, ↓ plasma osmolality → negative feedback
- Thirst: Osmoreceptors also activate thirst centres (lamina terminalis) at slightly higher osmolality threshold than ADH release; together ADH and thirst defend plasma osmolality within narrow range ($\pm 1\text{--}2\%$)

Clinical Note: *Syndrome of Inappropriate ADH (SIADH): ADH secreted despite low plasma osmolality → water retention → dilutional hyponatraemia. Causes: CNS disease, pulmonary disease, malignancy (ectopic ADH), drugs (SSRIs, carbamazepine, NSAIDs). Diagnosis: low Posm, urine Osm >100, urine Na >40. Treatment: fluid restriction ± hypertonic saline; vaptans (V2 receptor antagonists) in severe cases.*

C. The Renin-Angiotensin-Aldosterone System (RAAS)

While ADH primarily governs water reabsorption, RAAS primarily governs sodium (and therefore ECF volume) regulation, which in turn controls blood pressure and water distribution:

- Stimulus: ↓ Renal perfusion pressure (sensed by juxtaglomerular cells) → Renin secretion (also stimulated by ↑ sympathetic tone and ↓ NaCl delivery to macula densa)
- Cascade: Renin cleaves angiotensinogen (liver) → Angiotensin I → ACE (lung endothelium) → Angiotensin II (Ang II)
- Ang II actions: (1) Adrenal cortex (zona glomerulosa) → ↑ Aldosterone synthesis; (2) Renal vasoconstriction → ↓ GFR; (3) Hypothalamus → ↑ ADH release + thirst; (4) Direct Na^+ reabsorption in proximal tubule
- Aldosterone: Binds mineralocorticoid receptor (MR) in principal cells of collecting duct → ↑ transcription of ENaC (Na^+ channel) and ROMK (K^+ channel) → Na^+ in/ K^+ out → water follows Na^+ → expands ECF volume → ↑ blood pressure → negative feedback on renin
- ANP/BNP counter-regulatory: Released by atrial/ventricular cardiomyocytes in response to ↑ stretch (↑ volume) → inhibit renin, aldosterone, and ADH → natriuresis and diuresis → ↓ blood pressure

III. ELECTROLYTE PHYSIOLOGY

A. Sodium (Na^+) — The ECF Gatekeeper

Sodium is the primary determinant of ECF osmolality and therefore ECF volume. The body contains ~3,500–4,000 mEq total Na^+ ; ~85% is exchangeable. DRI: AI = 1,500 mg/day; Chronic Disease Risk Reduction Intake (CDRR) = 2,300 mg/day. Typical US intake: 3,400–3,600 mg/day.

Renal Handling of Na^+ (filtration–reabsorption balance):

- Proximal tubule: ~65–70% of filtered Na^+ reabsorbed via Na^+/H^+ exchanger (NHE3), Na^+ -glucose co-transporter (SGLT2), Na^+ -amino acid transporters, Na^+/K^+ -ATPase (basolateral). Driven by Ang II. SGLT2 inhibitors (gliflozins) block this segment — glycosuric and natriuretic effect
- Loop of Henle: ~25% via $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporter (NKCC2 — target of loop diuretics: furosemide); creates medullary hypertonicity for urine concentration
- Distal convoluted tubule: ~5% via Na^+/Cl^- co-transporter (NCC — target of thiazide diuretics)
- Collecting duct: ~1–3% via ENaC (epithelial Na^+ channel — regulated by aldosterone)

Disorder	Causes Clinical Features Nutritional Considerations
Hyponatraemia ($\text{Na}^+ < 135$ mEq/L)	Dilutional (SIADH, CHF, cirrhosis, excess hypotonic fluid); Depletional (losses > intake: vomiting, diarrhoea, Addison's, thiazide diuretics). Sx: nausea, headache, confusion, seizures ($\text{Na}^+ < 120$). Correct slowly (<8–10 mEq/L/24h) — rapid correction → osmotic demyelination syndrome (ODS/CPM)
Hypernatraemia ($\text{Na}^+ > 145$ mEq/L)	Water deficit ($\downarrow$ intake, diabetes insipidus, insensible losses, osmotic diuresis) or Na^+ gain (hypertonic fluids, salt tablets). Sx: thirst, lethargy, seizures (elderly, infants at risk). Correct slowly with hypotonic fluids (max 0.5 mEq/L/h). Enteral nutrition patients: ensure adequate free water

B. Potassium (K^+) — The ICF Maestro

The body contains ~3,500 mEq total K^+ , of which >98% is intracellular (~140 mEq/L ICF vs ~4 mEq/L ECF). This steep gradient, maintained by Na^+/K^+ -ATPase, is essential for cell membrane resting potential (Nernst equation) and therefore excitability of cardiac and skeletal muscle and neurones. Even small changes in ECF K^+ profoundly affect membrane potential.

K^+ Internal Distribution (Transcellular Shift):

- $\uparrow$ Insulin → activates Na^+/K^+ -ATPase → drives K^+ INTO cells (basis of insulin in hyperkalemia treatment)
- $\uparrow$ Catecholamines (β_2 adrenergic) → activate Na^+/K^+ -ATPase → K^+ into cells (stress, bronchodilators)
- Alkalosis → H^+ exits cells in exchange for K^+ → hypokalaemia; Acidosis → opposite → hyperkalaemia
- Insulin deficiency (DKA) + acidosis → hyperkalaemia despite total body K^+ depletion — critical clinical scenario

Renal K⁺ Excretion:

- Filtered freely at glomerulus; ~90% reabsorbed proximally and in loop (NKCC2). Fine regulation at principal cells of collecting duct: aldosterone → ↑ ROMK (apical K⁺ channel) → K⁺ secretion into tubular lumen. High luminal flow rate also drives K⁺ secretion

Dietary Sources & DRI:

- DRI: AI = 2,600 mg/day (women), 3,400 mg/day (men). Average US intake: ~2,600–2,800 mg/day — below recommendations
- Richest sources: Potatoes (926 mg/medium), avocado (728 mg/half), banana (422 mg), sweet potato (438 mg/medium), white beans (502 mg/½ cup), spinach (419 mg/cup cooked), salmon (534 mg/3 oz)

Disorder	Causes ECG Changes Nutritional Management
Hypokalaemia (K ⁺ < 3.5 mEq/L)	GI losses (vomiting — HCl loss → metabolic alkalosis → K ⁺ secretion; diarrhoea); Diuretics (loop, thiazide); Hypomagnesaemia (refractory unless Mg replaced first); Eating disorders (vomiting, laxative abuse). ECG: ↓ T waves, U waves, widened QRS. Dietary: ↑ K ⁺ foods; IV/oral KCl supplementation. Must correct Mg ²⁺ concurrently
Hyperkalaemia (K ⁺ > 5.5 mEq/L)	Renal failure (most common); ACE-I/ARBs + K-sparing diuretics; Addison's disease; DKA; cell lysis (rhabdomyolysis, tumour lysis). ECG: peaked T waves, PR prolongation, widened QRS, sine wave, VF. Dietary: strict K ⁺ restriction (<2,000–2,500 mg/day in CKD stage 4–5). Emergency: IV calcium gluconate (stabilize membrane), insulin + dextrose, sodium bicarbonate, kayexalate, dialysis

C. Chloride (Cl⁻) — The Overlooked Anion

Cl⁻ is the principal extracellular anion (95–107 mEq/L) and moves passively with Na⁺ to maintain electroneutrality. Key roles: gastric HCl synthesis (parietal cells; H⁺/K⁺-ATPase secretes H⁺ while Cl⁻ follows); chloride shift in RBCs (HCO₃⁻/Cl⁻ exchanger — CO₂ transport); CFTR channel mutations → cystic fibrosis (abnormal Cl⁻ transport → viscous secretions). Hypochloraemia occurs with vomiting (loss of HCl) or loop diuretics; hyperchloraemia with normal anion gap metabolic acidosis (diarrhoea, RTA, excessive NaCl infusion).

D. Calcium (Ca²⁺) — The Signaling Mineral

Total body Ca²⁺ ~1,000 g; 99% in bone (hydroxyapatite). Serum total Ca²⁺: 8.5–10.5 mg/dL (2.12–2.62 mmol/L). Three fractions: ionized (~45%, 1.1–1.35 mmol/L — biologically active), protein-bound (~40%, primarily albumin), complexed (~15%, with citrate, phosphate, bicarbonate). Always correct for albumin when interpreting total Ca²⁺:

Corrected Calcium Formula:

Corrected Ca²⁺ (mg/dL) = Measured Ca²⁺ + 0.8 × (4.0 - Serum Albumin g/dL)

Use ionized Ca²⁺ (iCa²⁺) measurement directly in acid-base disorders (pH affects protein binding: acidosis ↑ iCa²⁺; alkalosis ↓ iCa²⁺)

- PTH (parathyroid): $\uparrow$ when $iCa^{2+} \downarrow \rightarrow \uparrow$ bone resorption (osteoclasts via RANKL) + $\uparrow$ renal Ca^{2+} reabsorption (DCT) + $\uparrow$ calcitriol synthesis $\rightarrow$ net $\uparrow iCa^{2+}$
- Calcitriol ($1,25(OH)_2D$): $\uparrow$ intestinal Ca^{2+} absorption (TRPV6 channel, calbindin); synergises with PTH on bone
- Calcitonin (thyroid C cells): $\uparrow$ when $Ca^{2+} \uparrow \rightarrow$ inhibits osteoclasts $\rightarrow \downarrow$ bone resorption; minor physiological role in adults

Disorder	Causes Clinical Features Management
Hypocalcaemia ($iCa^{2+} < 1.1$ mmol/L)	Hypoparathyroidism (post-surgical most common); Vitamin D deficiency; Hypomagnesaemia (impairs PTH secretion and action); Acute pancreatitis; Rhabdomyolysis; Alkalosis ($\uparrow$ protein binding). Signs: Chvostek's sign (facial twitch), Trousseau's sign (carpal spasm), tetany, seizures, prolonged QT. Tx: Correct Mg^{2+} first; IV/oral Ca^{2+} + vitamin D supplementation
Hypercalcaemia ($iCa^{2+} > 1.35$ mmol/L)	Primary hyperparathyroidism (most common outpatient); Malignancy — bone mets, PTHrP (humoral hypercalcaemia of malignancy), myeloma; Vitamin D toxicity; Granulomatous disease (extra-renal calcitriol synthesis: sarcoidosis, TB). Signs: 'Bones, stones, groans, moans' (bone pain, renal stones, constipation, depression/psychosis). Treat underlying cause; hydration; bisphosphonates; calcitonin

E. Magnesium (Mg^{2+}) — The Forgotten Cation

Total body Mg^{2+} ~24 g; ~60% in bone, ~20% muscle, ~19% soft tissue, <1% plasma (0.75–0.95 mmol/L). The most important intracellular cation after K^+ (~10 mmol/L free intracellular). Required for all ATP-utilizing reactions (ATP exists as Mg -ATP), >300 enzyme reactions, DNA/RNA polymerases, and NMDA receptor gating. Serum Mg^{2+} is a poor marker of total body stores — normal serum Mg^{2+} can coexist with significant intracellular depletion.

- Hypomagnesaemia (< 0.75 mmol/L): Causes — alcohol use disorder (most common in hospitalized patients), diarrhoea/malabsorption, proton pump inhibitor (PPI) use ($\downarrow$ intestinal absorption via TRPM6), type 2 diabetes ($\uparrow$ renal Mg^{2+} wasting), loop/thiazide diuretics, refeeding syndrome. Features: neuromuscular irritability (tetany, tremor, seizures), cardiac arrhythmias, refractory hypokalaemia (Mg^{2+} required for renal K^+ retention — can't correct K^+ without correcting Mg^{2+}), refractory hypocalcaemia (Mg^{2+} needed for PTH secretion)
- Hypermagnesaemia (> 1.1 mmol/L): Rare; iatrogenic (Mg^{2+} supplementation, antacids/laxatives containing Mg^{2+}) in setting of renal failure. Features: $\downarrow$ DTRs, respiratory depression, cardiac arrest at high levels

F. Phosphate (PO_4^{3-}) — Energy & signaling Hub

Serum phosphate: 2.5–4.5 mg/dL (0.8–1.45 mmol/L). Major compartment: ~85% bone (hydroxyapatite with Ca^{2+}), ~14% intracellular. Critically important for: ATP synthesis, 2,3-DPG (O_2 delivery), phospholipid membranes, nucleic acids, protein phosphorylation signaling cascades. Regulated by FGF-23 (phosphatonin, from bone) and calcitriol.

- Hypophosphataemia (< 2.5 mg/dL): Refeeding syndrome (most critical nutritional cause — see below), malabsorption, vitamin D deficiency, hyperparathyroidism (PTH $\rightarrow$ phosphaturia), antacid abuse (Al^{3+}/Ca^{2+} bind phosphate), alcohol use disorder. Features: muscle weakness, respiratory failure (impaired diaphragm), haemolytic anaemia ($\downarrow$ RBC 2,3-DPG, ATP), encephalopathy, bone pain (osteomalacia)

- Hyperphosphataemia ($> 4.5 \text{ mg/dL}$): Primarily CKD ($\downarrow$ renal excretion + secondary hyperparathyroidism); hypoparathyroidism; tumour lysis syndrome. Causes metastatic calcification with Ca^{2+} ; dietary restriction to $<800 \text{ mg/day}$ + phosphate binders (calcium acetate, sevelamer, lanthanum carbonate) in CKD

Clinical Note: Refeeding Syndrome: *Introducing carbohydrate after prolonged starvation $\rightarrow$ insulin surge $\rightarrow$ massive intracellular shift of PO_4^{3-} , K^+ , and $\text{Mg}^{2+} \rightarrow$ severe deficiencies. Cardiac arrhythmias, respiratory failure, and death can result. Prevention: Identify high-risk patients (prolonged fasting >5 days, BMI <16 , unintentional weight loss $>15\%$), start nutrition slowly (10 kcal/kg/day), supplement PO_4^{3-} , K^+ , Mg^{2+} , thiamin (B1) before and during refeeding.*

IV. ACID-BASE BALANCE & pH REGULATION

A. Fundamentals of pH Chemistry

The pH of arterial blood is maintained within 7.35–7.45 (mean 7.40) — a remarkably narrow range representing only a 2-fold variation in $[\text{H}^+]$ (from 35 to 45 nmol/L). Even small deviations profoundly alter protein conformation, enzyme activity, and cellular excitability. The body produces $\sim 15,000$ – $20,000 \text{ mmol/day}$ of CO_2 (volatile acid, exhaled by lungs) and ~ 50 – 100 mEq/day of non-volatile acids (from protein metabolism — sulphuric acid from sulphur amino acids; phosphoric acid; organic acids), excreted by kidneys.

Henderson-Hasselbalch Equation:

$$\text{pH} = \text{pKa} + \log \left(\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \right)$$

pKa of $\text{H}_2\text{CO}_3 = 6.1$; at physiological pH 7.40: $[\text{HCO}_3^-] / [\text{H}_2\text{CO}_3] = 20:1$. CO_2 (dissolved) = $\text{PCO}_2 \times 0.0307$. Normal values: pH 7.35–7.45 | PaCO_2 35–45 mmHg | HCO_3^- 22–26 mEq/L

B. The Three Buffer Systems

1. Bicarbonate/Carbonic Acid Buffer System (Primary Extracellular)

This is the most important physiological buffer despite not being the most powerful chemically, because both components are regulated: lungs control CO_2 (PaCO_2) and kidneys control HCO_3^- . Together they provide rapid (minutes) and prolonged (hours-days) buffering.

CO_2 Buffering Reaction:



Carbonic anhydrase (CA) in RBCs, renal tubule, gastric mucosa catalyses this reaction ($10,000\times$ acceleration). CA inhibitor: acetazolamide ($\downarrow \text{HCO}_3^-$ reabsorption $\rightarrow$ metabolic acidosis).

2. Phosphate Buffer System (Primary Intracellular & Urine)

$\text{H}_2\text{PO}_4^- \rightleftharpoons \text{H}^+ + \text{HPO}_4^{2-}$ ($\text{pKa} = 6.8$ — optimal for physiological pH). Most important intracellular buffer and in urine (urinary phosphate titrates H^+ secreted by collecting duct, forming titratable acid — allows net H^+ excretion without extreme urine acidity). Low plasma phosphate concentrations limit plasma buffering contribution.

3. Protein Buffer System (Most Abundant by Capacity)

Plasma proteins (albumin — dominant) and intracellular proteins contribute ~60–70% of total body buffering capacity. Histidine residues (pKa ~6.0) and N-terminal amino groups are primary buffering sites. Haemoglobin is the principal blood buffer (Haldane effect: deoxyhaemoglobin is a better buffer than oxyhaemoglobin — explains why venous blood handles CO₂ load more effectively).

Hypoalbuminaemia (malnutrition, cirrhosis) reduces buffering capacity → greater vulnerability to acid-base disturbances.

C. Renal Mechanisms of H⁺ Regulation

The kidneys perform the definitive elimination of non-volatile acids and long-term regulation of plasma [HCO₃⁻]. Three renal mechanisms:

- HCO₃⁻ reabsorption (proximal tubule): ~85% of filtered HCO₃⁻ reclaimed via NHE3 (Na⁺/H⁺ exchanger) + carbonic anhydrase; not net acid excretion but prevents loss of existing buffer
- Titratable acid excretion (collecting duct): H⁺-ATPase secretes H⁺ into tubular lumen; H⁺ binds HPO₄²⁻ → H₂PO₄⁻ (titratable acid) — measured as titratable acidity; responsible for ~10–30 mEq/day net acid excretion
- Ammonium (NH₄⁺) excretion: Glutamine → NH₃ (glutaminase in proximal tubule) → NH₃ diffuses into tubular lumen → combines with secreted H⁺ → NH₄⁺ (trapped — cannot diffuse back); major pathway for net acid excretion, ~30–50 mEq/day; increases substantially in chronic acidosis (adaptive ↑ glutaminase activity)

D. Primary Acid-Base Disorders — Classification & Recognition

Acid-base disturbances are classified by the primary disorder (metabolic vs respiratory) and direction (acidosis vs alkalosis). Compensation is always in the same direction as the primary disorder and never fully corrects pH to normal (which would remove the stimulus for compensation).

Disorder	Primary Change pH Direction	Compensation Expected Response
Metabolic Acidosis	↓ HCO ₃ ⁻ (< 22 mEq/L) pH < 7.35	Respiratory: ↑ ventilation → ↓ PaCO ₂ Winters' formula: Expected PaCO ₂ = 1.5 × [HCO ₃ ⁻] + 8 ± 2
Metabolic Alkalosis	↑ HCO ₃ ⁻ (> 26 mEq/L) pH > 7.45	Respiratory: ↓ ventilation → ↑ PaCO ₂ Expected PaCO ₂ = 0.7 × [HCO ₃ ⁻] + 21 ± 2
Respiratory Acidosis	↑ PaCO ₂ (> 45 mmHg) pH < 7.35	Renal: ↑ HCO ₃ ⁻ reabsorption + ammoniogenesis Acute: ΔHCO ₃ ⁻ = 0.1 × ΔPCO ₂ ; Chronic: ΔHCO ₃ ⁻ = 0.35 × ΔPCO ₂
Respiratory Alkalosis	↓ PaCO ₂ (< 35 mmHg) pH > 7.45	Renal: ↓ HCO ₃ ⁻ reabsorption → ↑ HCO ₃ ⁻ excretion Acute: ΔHCO ₃ ⁻ = 0.2 × ΔPCO ₂ ; Chronic: ΔHCO ₃ ⁻ = 0.5 × ΔPCO ₂

Anion Gap — Key Diagnostic Tool in Metabolic Acidosis:

Anion Gap (AG) Calculation:

$AG = [Na^+] - ([Cl^-] + [HCO_3^-])$ | Normal: 8–12 mEq/L (3–11 with albumin correction)

Correct for albumin: $AG (corrected) = AG (measured) + 2.5 \times (4.0 - \text{Albumin g/dL})$

High AG Metabolic Acidosis (MUDPILES)	Normal AG Metabolic Acidosis (hyperchloraemic)
M — Methanol poisoning (→ formic acid)	Diarrhoea (HCO_3^- loss from GI tract)
U — Uraemia/renal failure	Renal tubular acidosis (RTA Types 1, 2, 4)
D — DKA / starvation ketoacidosis	Acetazolamide, topiramate (CA inhibition)
P — Propylene glycol; Paracetamol (late)	Ileostomy / ureterosigmoidostomy
I — Isoniazid / Iron toxicity	Pancreatic fistula (HCO_3^- rich secretions)
L — Lactic acidosis (Type A: ↓ O_2 delivery; Type B: metformin, thiamin deficiency)	Rapid NaCl infusion ('dilutional acidosis')
E — Ethylene glycol (→ oxalic acid)	Addison's disease (hypoadosteronism)
S — Salicylates (mixed resp alkalosis + met acidosis)	Renal failure with HCO_3^- wasting

Clinical Note: Thiamin (B1) deficiency → impaired pyruvate dehydrogenase → pyruvate cannot enter TCA → pyruvate → lactate → Type B lactic acidosis. Clinically important in: refeeding syndrome, alcohol use disorder, critically ill patients receiving prolonged glucose infusions without thiamin. Always administer IV thiamin before glucose in at-risk patients.

Causes of Metabolic Alkalosis (For Clinical Practice):

- Chloride-responsive (urine $Cl^- < 20$ mEq/L — 'contraction alkalosis'): Vomiting / NG suction (HCl loss → HCO_3^- retention + volume contraction → RAAS activation → further HCO_3^- retention); loop/thiazide diuretics. Treatment: volume repletion with $NaCl \pm KCl$
- Chloride-resistant (urine $Cl^- > 20$ mEq/L): Primary hyperaldosteronism (Conn's syndrome); Cushing's syndrome; Bartter's/Gitelman's syndromes; severe hypokalaemia. Treatment: address underlying cause

V. FLUID & ELECTROLYTE ASSESSMENT & CLINICAL NUTRITION APPLICATION

A. Clinical Assessment of Fluid Status

Assessment Method	Parameters & Clinical Significance
Hydration history	Fluid intake (24h recall), urine frequency and color (pale yellow = adequate; dark = dehydrated; colorless = overhydrated), thirst, recent weight change (acute weight loss/gain reflects fluid shifts)
Physical examination	Skin turgor (pinch test — delayed recoil in dehydration; unreliable in elderly), mucous membrane moisture, orthostatic vital signs ($\uparrow$ HR >20 bpm or $\downarrow$ SBP >20 mmHg on standing = significant volume depletion), peripheral oedema (pitting vs non-pitting), JVD (jugular venous distension — $\uparrow$ in fluid overload, $\downarrow$ in dehydration), capillary refill time
Urine markers	Urine specific gravity (1.001–1.035; >1.020 = concentrated/dehydrated); urine osmolality (100–1,200 mOsm/kg; <100 = ADH suppressed; >800 = ADH active); urine Na^+ (<20 mEq/L = renal Na^+ conservation = volume depleted; >40 = renal loss); FENa = $(\text{urine Na} \times \text{serum Cr}) / (\text{serum Na} \times \text{urine Cr}) \times 100$ ($<1\%$ = prerenal; $>2\%$ = intrinsic renal)
Laboratory markers	Serum Na^+ (osmolality), BUN: creatinine ratio ($>20:1$ = prerenal dehydration), serum albumin (oncotic pressure, chronic malnutrition), haematocrit (elevated in dehydration), serum electrolytes panel, arterial blood gas (pH, PaCO_2 , HCO_3^- — full acid-base assessment)
Body weight monitoring	Daily weight in hospitalized/ICU patients; acute changes >0.5 – 1 kg/day reflect fluid shifts, not true tissue changes; weight trend over weeks = tissue/nutritional change

B. Nutritional Considerations in Specific Clinical Scenarios

1. Chronic Kidney Disease (CKD)

- Fluid restriction: Typically not required until advanced CKD (stage 5/dialysis) if urine output maintained; anuric dialysis patients typically restricted to 1,000–1,500 mL/day fluid intake
- Sodium: Restrict to 2,000–2,300 mg/day (controls hypertension and fluid retention); hidden sodium in processed foods — detailed label reading counselling essential
- Potassium: Restrict in stage 4–5 CKD ($< 2,000$ – $2,500$ mg/day); monitor serum K^+ closely with RAAS blockade; leaching vegetables (boiling, discarding water) reduces K^+ by $\sim 50\%$ — allows safer vegetable inclusion
- Phosphate: Restrict to 800–1,000 mg/day in stage 3b+ CKD; avoid organic phosphate additives in processed foods (highly bioavailable vs natural food phosphate); phosphate binders with meals as prescribed
- Calcium: Avoid over-supplementation $\rightarrow$ vascular calcification risk; dietary Ca^{2+} from food preferred; monitor corrected $\text{Ca}^{2+} \times \text{phosphate product}$ ($<55 \text{ mg}^2/\text{dL}^2$ to reduce calcification risk)

2. Exercise & Sport Nutrition

- Sweat composition: Volume 0.5–2 L/hour (trained athletes); Na^+ 20–80 mEq/L (highly individual — 'salty sweaters' prone to hyponatraemia); K^+ ~5 mEq/L; Mg^{2+} ~0.3 mEq/L; Cl^- 30–70 mEq/L
- Hyponatraemia of exercise: Excessive hypotonic fluid (plain water) ingestion during endurance events → dilutional hyponatraemia (Exercise-Associated Hyponatraemia, EAH); potentially fatal; prevent by drinking to thirst rather than scheduled volumes; electrolyte beverages for >2 hour events
- Dehydration thresholds: >2% body weight loss → impaired cognitive and physical performance; >3% → thermoregulatory impairment; >5% → heat exhaustion risk
- Hydration strategy: Pre-exercise: 5–7 mL/kg 4 hours before; During: 150–350 mL/15–20 min (adjust for sweat rate); Post-exercise: 1.5 L per kg body weight lost (sodium-containing beverage to facilitate rehydration and prevent subsequent urinary losses)

Counseling Tip: *For clinical practice: Urine color chart is the most practical hydration monitoring tool for patients — pale straw yellow (color 1–3 on 8-point scale) indicates adequate hydration. Simple, free, and surprisingly effective for patient self-monitoring.*

3. Nutrition Support & Electrolyte Management

- Enteral nutrition (EN): Monitor free water balance (most tube feeds ~75–85% water; additional free water flushes required to prevent hypernatraemia, especially in cognitively impaired or sedated patients who cannot express thirst)
- Parenteral nutrition (PN): Daily electrolyte monitoring (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , PO_4^{3-}) during initiation; adjust formulation based on trends; standard PN electrolyte requirements: Na^+ 1–2 mEq/kg/day, K^+ 1–2 mEq/kg/day, Ca^{2+} 10–15 mEq/day, Mg^{2+} 8–20 mEq/day, PO_4^{3-} 20–40 mmol/day
- Refeeding syndrome prevention (NICE criteria): Identify high-risk patients; start at ≤ 10 kcal/kg/day; increase over 4–7 days; supplement thiamin 200–300 mg TID before and for first 10 days; monitor and replace PO_4^{3-} , K^+ , Mg^{2+} aggressively; restrict fluid and sodium in first few days

Lecture 1 Summary — Key Principles

- TBW ~60% in men, ~50% in women; ICF (K^+ -rich, ~25 L) and ECF (Na^+ -rich, ~17 L); water moves via osmosis through AQP channels driven by osmolality gradients
- Water homeostasis: ADH ($\text{V}_2/\text{AQP2}$ axis — water reabsorption) + RAAS (aldosterone/ENaC — Na^+ and volume) + ANP/BNP (counter-regulation); thirst activates at slightly higher osmolality than ADH threshold
- Starling forces govern capillary-interstitial fluid exchange; oedema = disruption of hydrostatic-oncotic balance (hypoalbuminaemia, ↑ capillary pressure, ↑ permeability)
- Electrolytes: Na^+ (ECF volume/osmolality, RAAS-regulated), K^+ (membrane potential, insulin/aldosterone-regulated — refractory hypokalaemia → check Mg^{2+} first), Ca^{2+} (PTH/calcitriol triad — correct for albumin), Mg^{2+} (>300 enzymes — serum is poor marker), PO_4^{3-} (ATP, 2,3-DPG — critical in refeeding)
- Three buffer systems: bicarbonate/ CO_2 (regulated by lungs + kidneys — most clinically important), phosphate (intracellular + urine), proteins/haemoglobin (greatest total capacity)
- Acid-base: four primary disorders (metabolic/respiratory × acidosis/alkalosis); $\text{AG} = \text{Na}^+ - (\text{Cl}^- + \text{HCO}_3^-)$; high-AG causes = MUDPILES; normal-AG = hyperchloraemic (GI/renal HCO_3^- loss)
- Critical clinical pearls: Refeeding syndrome (PO_4^{3-} , K^+ , Mg^{2+} , thiamin depletion), Hypomagnesaemia → refractory hypokalaemia + hypocalcaemia, SIADH management (fluid restriction, slow correction), Exercise hyponatraemia (drink to thirst)

Suggested Reading:

1. Halperin ML & Kamel KS (2010). Fluid, Electrolyte and Acid-Base Physiology: A Problem-Based Approach, 4th Ed. |
2. Rose BD & Post TW (2001). Clinical Physiology of Acid-Base and Electrolyte Disorders, 5th Ed.
3. NICE Guidelines NG22 (2020): Nutrition Support for Adults. |
4. Kregenow DA & Swenson ER (2020). Respiratory acid-base physiology. Respir Physiol Neurobiol.

LECTURE 2

Body Composition

Models, Lifespan Changes, Measurement Techniques & Clinical Assessment

Nutrition & Dietetics MS Program | Body Regulation & Composition Series

Learning Outcomes — By the end of this lecture, students will be able to:

- Explain the two-compartment, four-compartment, and multi-compartment models of body composition
- Describe the physiological determinants of fat mass, fat-free mass, muscle mass, bone mineral, and total body water
- Trace changes in body composition across the full lifespan from infancy through advanced old age
- Compare the principles, accuracy, limitations, and appropriate clinical applications of major body composition measurement techniques
- Interpret body composition assessment data in clinical settings and apply findings to nutritional intervention planning
- Identify disease-associated alterations in body composition: sarcopenia, cachexia, sarcopenic obesity, and lipodystrophy

I. BODY COMPOSITION MODELS

A. The Compartment Framework

Body composition refers to the proportions of anatomical and chemical components that make up total body mass. Analysis can be performed at multiple levels of organization — atomic, molecular, cellular, tissue-organ, and whole-body, and using different compartment models. Each model makes specific assumptions that determine its accuracy and limitations.

Model	Compartments Assumptions Clinical Utility
2-Compartment (2C) Model	Fat Mass (FM) + Fat-Free Mass (FFM). Foundational model; FFM assumed to have fixed density (1.1 kg/L), water content (73.2%), and mineral content. Simplest; basis of hydrostatic weighing and skinfold equations. Limitation: assumptions of fixed FFM composition inaccurate in extremes of age, disease, obesity, and athletes
3-Compartment (3C) Model	FM + Total Body Water (TBW) + Residual FFM (protein + mineral). Removes hydration assumption by directly measuring TBW (deuterium/tritium dilution). More accurate in elderly and athletes. Still assumes mineral content of FFM
4-Compartment (4C) Model	FM + TBW + Bone Mineral (BM) + Protein. The gold standard reference model. Simultaneously measures body volume (underwater weighing or Bod Pod), TBW (isotope dilution), and bone mineral (DXA). No assumption

	about FFM composition. Most accurate; requires multiple techniques — research setting
Multi-compartment / Imaging Models	Separates visceral adipose tissue (VAT) from subcutaneous adipose tissue (SAT), individual organs, specific muscle groups, bone marrow. Requires CT/MRI. Highest specificity, differentiates metabolically harmful VAT from relatively benign SAT; used in obesity and metabolic disease research

B. Key Body Composition Components — Definitions & Physiological Significance

- **Fat Mass (FM):** All extractable lipid, essential fat (cell membranes, myelin, organs; ~3% men, ~12% women) + storage fat (triglycerides in adipocytes, subcutaneous and visceral). Essential fat required for hormonal function, neurological integrity, and reproductive health. Storage fat is the primary energy reserve (~7,700 kcal/kg). Reference range: men 10–20%, women 18–28% (age-dependent)
- **Fat-Free Mass (FFM):** Everything except fat — muscle, bone, organs, connective tissue, blood, and water. Tightly correlates with resting energy expenditure (REE ~21.5 kcal/kg FFM). Most metabolically active component. Decline of FFM (especially muscle) with aging drives the decline in REE
- **Skeletal Muscle Mass (SMM):** Largest component of FFM (~40% body weight in healthy young men). Site of ~80% of postprandial glucose disposal (insulin-dependent); major determinant of physical function and longevity; primary amino acid reservoir during catabolism. Appendicular Skeletal Muscle Mass (ASM = limb muscle) measured by DXA — used to diagnose sarcopenia
- **Bone Mineral Content (BMC) & Density (BMD):** Hydroxyapatite mineral (~60% Ca^{2+} , ~17% phosphate). BMC measured in grams; BMD (g/cm^2) = BMC/area. T-score = SD difference from young adult reference (peak BMD). $T \leq -2.5$ = osteoporosis; -2.5 to -1.0 = osteopenia. Peak BMD at ~25–30 years
- **Visceral Adipose Tissue (VAT):** Intra-abdominal fat surrounding organs. Metabolically highly active — secretes adipokines ($\uparrow$ IL-6, TNF- α , resistin; $\downarrow$ adiponectin), free fatty acids into portal circulation → hepatic IR. Strong predictor of insulin resistance, T2DM, CVD, and metabolic syndrome — independent of BMI. Measured by CT/MRI (gold standard) or estimated by waist circumference or DXA

II. BODY COMPOSITION ACROSS THE LIFESPAN

A. Foetal Development & Birth

Body composition changes dramatically during foetal development, reflecting organ maturation and accumulation of energy reserves:

- **First trimester (0–12 weeks):** Primarily organogenesis; minimal fat deposition; ~95% water, ~5% fat at 12 weeks
- **Second trimester (13–26 weeks):** Skeletal muscle and organ growth; fat begins accumulating (~3% at 20 weeks); bone mineralization accelerates from week 22–25 (peak accretion rate)
- **Third trimester (27–40 weeks):** Rapid adipose tissue accumulation — fat increases from ~3% (26 weeks) to ~14–16% at term (40 weeks). 80% of total bone mineral laid down in this trimester. Weight gain: ~200 g/week in third trimester

- At birth (term newborn): Fat ~14–16% of body weight; TBW ~75–80%; skeletal muscle ~25%. Premature infants have markedly reduced fat stores and bone mineral — critical nutritional vulnerability

Clinical Note: *Intrauterine Growth Restriction (IUGR) and preterm birth result in altered body composition — proportionally less muscle and more fat (programming of metabolic dysfunction).*

These infants have ↑ risk of obesity, insulin resistance, and CVD in adulthood (Barker/Developmental Origins of Health and Disease hypothesis). Catch-up growth characterized by disproportionate fat gain further amplifies metabolic risk.

B. Infancy & Early Childhood (0–5 Years)

- Infancy (0–12 months): Rapid growth; fat mass increases from 14% (birth) to peak ~25–27% at 4–6 months (adiposity rebound preparation); TBW decreases from ~75% to ~65%; brain growth consumes disproportionate energy (brain uses ~60–70% of total energy in infants)
- Toddler adiposity rebound (2–7 years): After declining from 6-month peak, fat percentage rises again at ~5.5–6 years (adiposity rebound). Early adiposity rebound (before age 5) is strongly predictive of adult obesity risk
- Muscle mass accretion: Gradual linear growth with body length; relatively constant proportion of FFM throughout early childhood; skeletal muscle growth driven by growth hormone (GH) and IGF-1 axis
- Bone mineralization: Linear with height; positive calcium balance essential; human milk vs formula: human milk better calcifies per bone volume (IGF-1, lactoferrin) despite lower Ca²⁺ content — bioavailability differences

C. School Age & Adolescence (6–19 Years)

Puberty is the most dramatic period of body composition change outside of foetal life. The timing, magnitude, and pattern differ markedly by sex:

Phase	Males	Females
Puberty onset (Tanner staging)	Mean age 11.5 years (range 9–14)	Mean age 10.5 years (range 8–13)
Growth velocity peak (PHV)	~10 cm/year; age 13.5 years	~9 cm/year; age 11.5 years; precedes menarche
Muscle mass change	↑↑↑ Dramatic: testosterone → ↑ IGF-1 → muscle protein synthesis; ↑ from ~42% to ~54% FFM (boys add ~33 kg lean mass)	Modest: ↑ muscle mass but less than boys; ↑ from ~45% to ~48% FFM (girls add ~16 kg lean mass)
Fat mass change	↓ Relative: fat % ↓ from ~18% → 14–18% (muscle gain dilutes fat %)	↑↑ Marked: oestrogen drives adipose deposition (gluteal, femoral, breast); fat ↑ from ~22% → 25–28%; gynoid distribution
Bone mineral accretion	Peak bone mineral accrual at ~14 years; 37% of peak bone mass gained during adolescence; testosterone → ↑ periosteal growth → larger, stronger bones	Peak accrual at ~12–13 years; oestrogen → endosteal apposition; smaller cross-section than males

Clinical Note: *Peak Bone Mass (PBM) and the Adolescent Window: ~90% of peak bone mass is established by age 18. Nutritional adequacy during puberty (calcium 1,300 mg/day, vitamin D 600 IU/day, protein, phosphorus) is critical and irreversible — suboptimal adolescent nutrition sets a permanent lower peak bone mass trajectory with lifelong implications for osteoporosis risk. Calcium supplementation in adolescent girls with low intake significantly increases BMD (Johnston et al., NEJM 1992).*

D. Young Adulthood (20–39 Years)

Body composition is at its lifetime optimum early in this period, then begins a slow decline:

- Peak muscle mass: ~25–30 years for men; ~20–25 years for women. After peak, gradual loss begins (~0.5–1% per year) even in healthy sedentary adults — accelerates with inactivity
- Peak bone mineral density (BMD): ~25–30 years. After this peak, net bone loss begins (bone resorption > bone formation). Rate of loss accelerates markedly at menopause
- Fat mass trajectory: Tends to increase slowly with age even if body weight is stable — FM increases as FFM decreases ('adiposity creep'). This shift is clinically significant as BMI may appear stable while body composition worsens
- Visceral fat begins accumulating: Even in weight-stable individuals, VAT gradually increases from the late 20s — associated with progressive metabolic risk even before frank obesity

E. Middle Adulthood (40–65 Years)

This decade is characterized by accelerating composition deterioration and represents the critical window for preventive intervention:

- Accelerated muscle loss: Sarcopenic processes become clinically apparent; ~1–2% skeletal muscle mass loss/year after 50; accompanied by loss of type II (fast-twitch) muscle fibres preferentially — impacts power and functional strength more than endurance
- Hormonal decline contributes substantially: Testosterone declines ~1–2%/year in men after 30 (andropause); growth hormone pulsatility and IGF-1 decline; menopause (average age 51) → rapid oestrogen decline → accelerated bone loss (2–5% BMD/year in first 5–7 post-menopausal years) + redistribution of fat from gynoid (gluteal-femoral) to android (abdominal/visceral) pattern
- Metabolic consequences: ↓ muscle mass → ↓ REE → positive energy balance tendency → weight gain predominantly as fat; ↑ VAT → ↑ insulin resistance → ↑ T2DM risk; ↑ ectopic fat deposition (liver — NAFLD; muscle — intermuscular adipose tissue, IMAT — further impairs insulin sensitivity)

Clinical Note: *'Normal weight obesity' (NWO): Individuals with normal BMI but elevated fat mass (>30% women, >25% men) and normal or low lean mass — increasingly recognized in middle age, especially in sedentary women. BMI fails to identify this group. Associated with metabolic syndrome, hypertension, and CVD risk despite 'healthy' BMI. Body composition assessment (DXA, BIA) is essential.*

F. Older Adulthood (65+ Years) — Sarcopenia, Frailty & Osteoporosis

Advanced ageing is characterized by progressive, clinically significant losses in muscle and bone that substantially increase morbidity, disability, and mortality risk:

Sarcopenia — Definition & Criteria (EWGSOP2, 2019):

Sarcopenia = low muscle strength (grip strength < 27 kg men / < 16 kg women as primary indicator) + low muscle quantity/quality (DXA: ASM/height² < 7.0 kg/m² men / < 5.5 kg/m² women). Severe sarcopenia = above + low physical performance (gait speed < 0.8 m/s or SPPB ≤ 8).

Mechanism of Sarcopenia	Clinical Implication
↓ Satellite cell activation and muscle protein synthesis (↓ mTORC1 response to leucine and resistance exercise)	↑ Protein requirements in elderly (1.0–1.2 g/kg/day, vs 0.8 g/kg/day RDA); leucine-rich protein sources (whey, eggs, soy)
↓ Motor neuron number and NMJ integrity → denervation atrophy	Type II fibre loss → impaired power, balance → ↑ fall risk
↑ Inflammatory cytokines (IL-6, TNF-α) → muscle protein catabolism (NF-κB → ubiquitin-proteasome)	Anti-inflammatory diet (Mediterranean) may attenuate; ↑ omega-3 (EPA/DHA) stimulates muscle protein synthesis
↓ Growth hormone and IGF-1 — impaired anabolic signaling	HMB (β-hydroxy β-methylbutyrate) supplementation may partially compensate mTORC1 activation
Anabolic resistance — blunted MPS response to protein intake	Distribute protein evenly across 3–4 meals (25–30g per meal) rather than concentrated in one meal
↓ Physical activity → disuse atrophy	Resistance training 2–3×/week is most potent intervention; synergistic with adequate protein
Mitochondrial dysfunction, ↑ intramyocellular lipid (IMCL)	↑ oxidative stress within muscle; associated with insulin resistance

Cachexia vs Sarcopenia vs Frailty:

Condition	Primary Driver	Distinguishing Feature
Sarcopenia	Aging + inactivity	Muscle mass/strength/function loss; body weight may be stable or increased if fat preserved
Cachexia	Disease (cancer, CHF, CKD, COPD, HIV) + inflammation	Involuntary weight loss + muscle wasting; driven by inflammatory cytokines (IL-6, TNF-α) + ↑ catabolism; NOT fully reversible with nutrition alone
Frailty	Multi-system decline	Fried criteria: weight loss, exhaustion, low activity, slow gait, weak grip — 3+ = frail; overlap with sarcopenia; ↑ mortality, hospitalization, disability

Osteoporosis:

- Pathophysiology: Imbalance of osteoclast (bone resorption) vs osteoblast (bone formation) activity. Post-menopausal: ↓ oestrogen → ↑ RANKL expression → ↑ osteoclast differentiation/activity; Senile: ↓ Ca^{2+} absorption, ↓ calcitriol, ↑ PTH → secondary hyperparathyroidism → cortical bone loss
- Fracture risk: Hip fracture 1-year mortality up to 20–30% in elderly; vertebral fractures → height loss, kyphosis, restrictive lung disease, pain, depression
- Nutritional intervention: Calcium (1,000–1,200 mg/day from food preferentially; Ca-citrate better absorbed than Ca-carbonate in achlorhydria); Vitamin D (800–1,000 IU/day to achieve $25(\text{OH})\text{D} \geq 50 \text{ nmol/L}$); Protein ($\geq 1.0 \text{ g/kg/day}$ — higher protein ↑ IGF-1, ↑ Ca^{2+} absorption, ↑ bone formation); Vitamin K2 (MK-7, 90–180 µg/day — ↑ osteocalcin carboxylation); Magnesium; limit excess alcohol and tobacco

III. BODY COMPOSITION MEASUREMENT TECHNIQUES

A. Reference/Laboratory Methods

1. Hydrostatic (Underwater) Weighing — Historical Gold Standard

Principle: Based on Archimedes' principle — body density (D_b) = body mass / body volume; body volume = loss of weight underwater / water density. FM and FFM derived from density using Siri (1956) or Brozek equations. Assumes constant FFM density (1.1 kg/L) — a major source of error in non-reference populations.

Siri Equation (2C Model):

$$\% \text{ Body Fat} = (495 / D_b) - 450$$

Limitations: Residual lung volume must be measured; uncomfortable for some; 2C assumption; overestimates FM in Black individuals (higher bone density → higher D_b than assumed)

2. Air Displacement Plethysmography (ADP / Bod Pod)

Principle: Same as hydrostatic weighing but uses air displacement rather than water. Subject sits in sealed chamber; pressure-volume relationships (Boyle's Law) measure body volume. Body density → FM and FFM via 2C model equations. Advantages: Non-invasive, comfortable, rapid (5 min), valid in diverse populations (elderly, obese, children, pregnant). Corrections applied for surface area artefact (air trapped in clothing/hair) and thoracic gas volume (lung air). Better than underwater weighing for compliance. Same 2C limitations.

3. Dual-Energy X-Ray Absorptiometry (DXA)

Principle: X-ray beam at two energies (typically 40 and 70 keV) differentially attenuated by fat, lean mass, and bone mineral. Regional and total body analysis: Fat Mass, Lean Soft Tissue (LST ≈ FFM – BMC), Bone Mineral Content (BMC), and Bone Mineral Density (BMD). Can separate regional body regions (trunk, arms, legs) and estimate VAT (using dedicated software).

- Strengths: 3-compartment capability; regional analysis (essential for sarcopenia diagnosis — appendicular lean mass); BMD measurement with T-score; low radiation (~1–10 µSv, equivalent to <1 day background); reproducible; applicable across lifespan; widely available
- Limitations: Cannot distinguish intramuscular fat (IMAT) from lean tissue; hydration sensitive (oedema → overestimates lean mass); results differ between scanner manufacturers (Hologic vs

GE Lunar — not interchangeable); cannot perform in pregnancy; requires stable repositioning for serial scans

- Clinical applications: Sarcopenia diagnosis (EWGSOP2, ISCD criteria); osteoporosis staging; obesity phenotyping; monitoring changes in nutritional interventions; paediatric body composition (NHANES reference data for children)

Assessment Pearl: DXA ASM Index (ASMI) for sarcopenia: $ASMI = (\text{Arm lean mass} + \text{Leg lean mass}) / \text{Height}^2$. Low ASMI thresholds: men $< 7.0 \text{ kg/m}^2$, women $< 5.5 \text{ kg/m}^2$ (EWGSOP2). Combine with grip strength and gait speed for full sarcopenia staging.

4. Isotope Dilution — TBW Measurement

Principle: Orally administered isotopically labelled water (deuterium oxide $^2\text{H}_2\text{O}$ or O-18 enriched water ^{18}O -water) equilibrates with total body water over 3–5 hours. Dilution of known dose by measured enrichment in urine, saliva, or breath → TBW calculation. TBW → FFM (assuming 73.2% hydration of FFM — valid in hydrated adults). Highly accurate (± 1 –2%). Reference standard for TBW. Essential component of 4C model. Limited to research settings; expensive; requires mass spectrometry analysis.

B. Clinical Field Methods

5. Bioelectrical Impedance Analysis (BIA)

Principle: Alternating electrical current (50 kHz typically) passed through body; fat (poor conductor — high impedance) vs lean mass (high water/electrolyte content — low impedance). Impedance (Z) = Resistance (R) + Reactance (Xc). TBW, FFM, FM derived from impedance using prediction equations validated against criterion methods.

BIA Technology	Description & Clinical Use
Single-frequency BIA (SF-BIA)	50 kHz; most common; hand-to-foot or segmental; estimates TBW and FFM; reliable in healthy populations; significant error in disease states (oedema, dehydration)
Multi-frequency BIA (MF-BIA)	Multiple frequencies (1–500 kHz); estimates intracellular and extracellular water separately; better in clinical populations; basis for phase angle assessment
Bioelectrical Impedance Spectroscopy (BIS)	Full frequency spectrum; theoretically superior; models Cole-Cole — distinguishes ICW from ECW; used in fluid management research and dialysis
Phase Angle (PhA)	Derived from BIA ($\text{PhA} = \arctan[Xc/R] \times 180/\pi$); reflects cell membrane integrity and intracellular water; independent predictor of nutritional status, mortality in cancer, CKD, HIV, critical illness. Normal PhA: men $\sim 7.0^\circ$, women $\sim 6.5^\circ$; low PhA ($< 5^\circ$) = malnutrition, poor prognosis

Clinical Note: BIA Confounders — Must standardize: no food or fluid for 4 hours; no exercise for 12 hours; void bladder; no alcohol for 24 hours; standardize time of day (body water shifts ~ 1 –2 L diurnally); avoid in pregnancy, electronic implants (pacemakers), acute illness with altered fluid distribution. Oedema grossly overestimates lean mass by BIA — use with caution in CHF, CKD, cirrhosis.

6. Anthropometric Methods

Anthropometry uses external body measurements to estimate body composition. Low cost, no equipment beyond a scale and tape measure, widely applicable in field and clinical settings. Less precise than imaging or reference methods but highly practical:

Measure	Technique Reference Values Limitations
Body Mass Index (BMI)	Weight (kg) / Height ² (m ²). WHO: Underweight <18.5; Normal 18.5–24.9; Overweight 25–29.9; Obese ≥30 (Grade I 30–34.9; II 35–39.9; III ≥40). Cannot distinguish FM from FFM — same BMI can represent very different body compositions. Asian populations: ↑ metabolic risk at BMI ≥23 (adjusted WHO/IOTF thresholds). Athletes: BMI overestimates adiposity due to high muscle mass
Waist Circumference (WC)	Measured at umbilicus (WHO) or midpoint between lowest rib and iliac crest (NHANES). High risk: men >102 cm (>88 cm WHO); women >88 cm (>80 cm WHO). Asian cut-offs lower: men >90 cm, women >80 cm. Best anthropometric predictor of VAT and metabolic risk. Simple, reproducible with training. Affected by posture and breathing pattern
Waist-to-Hip Ratio (WHR)	WC / Hip circumference (widest point). High risk: men >0.95; women >0.85. Distinguishes android (apple) from gynoid (pear) fat distribution. Some evidence superior to WC for CVD risk prediction in women
Waist-to-Height Ratio (WHtR)	WC / Height. Universal cut-off proposed: >0.5 (equal sensitivity to WC for cardiometabolic risk in all ethnicities and sexes). Rule: 'Keep your waist less than half your height'
Skinfold Thickness	Callipers measure subcutaneous fat at standardized sites (Jackson-Pollock 3-site and 7-site; Durnin-Womersley 4-site; ISAK protocol). Equations predict body density → % fat (2C model). Sources of error: technique-dependent (require training and standardization, SEE ±3–4%), 2C model assumptions, site selection, calliper calibration, ethnicity
Mid-Upper Arm Circumference (MUAC)	Measured at mid-point of upper arm. Surrogate of muscle mass; used in malnutrition screening (MUAC < 21 cm in adults = severe malnutrition in humanitarian settings; specific paediatric references). Fast, non-invasive, requires only tape

7. CT & MRI Imaging

Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) provide gold-standard assessment of tissue distribution, including direct quantification of VAT, SAT, individual organs, and specific muscle groups. Single cross-sectional CT at L3–L4 vertebral level correlates closely with total VAT and SAT volume. CT uses Hounsfield Unit (HU) thresholds to distinguish fat (–30 to –190 HU) from muscle (–29 to +150 HU). MRI avoids ionizing radiation (preferred for serial measurements and research). Whole-body MRI can quantify total adipose tissue volume with high precision.

- L3 skeletal muscle index (L3-SMI): Cross-sectional area of skeletal muscle at L3 on CT, normalized to height² (cm²/m²); low L3-SMI thresholds: men < 52.4 cm²/m², women < 38.5 cm²/m² — defines CT sarcopenia; strong predictor of surgical complications, chemotherapy toxicity, and mortality in oncology
- Liver fat (hepatic steatosis): MRI-PDFF (Proton Density Fat Fraction) — gold standard for liver fat quantification; CT HU threshold < 40 HU suggests steatosis; FibroScan (vibration-controlled transient elastography) + CAP (Controlled Attenuation Parameter) — non-invasive liver fat and fibrosis assessment

Clinical Note: *Myosteatorsis (Intramuscular/Intermuscular Adipose Tissue — IMAT): Identified by low mean muscle HU on CT (<41 HU in men, <33 HU in women) or elevated IMCL on MR spectroscopy. Even with preserved muscle mass, myosteatorsis predicts poor functional outcomes, insulin resistance, and post-surgical complications. Often co-occurs with sarcopenic obesity.*

8. Ultrasound

Point-of-care ultrasound (POCUS) for body composition is rapidly expanding as an accessible, radiation-free, real-time bedside tool:

- Subcutaneous fat: B-mode ultrasound measures adipose layer thickness at standardized sites; good correlation with skinfolds; less operator-dependent
- Muscle thickness and cross-sectional area: Rectus femoris and quadriceps ultrasound assess muscle quantity and echogenicity (↑ echogenicity = ↑ fat infiltration/myosteatorsis = muscle quality ↓); biceps, tibialis anterior also studied. Validated against MRI in critical illness monitoring
- Advantages: Bedside in ICU, real-time, no radiation, inexpensive, portable, increasingly standardized protocols (EURO-MUSCULUS, SARCUS)

IV. COMPARISON OF METHODS — CLINICAL SELECTION GUIDE

Method	Accuracy	Practical Utility	Best Clinical Use
4-Compartment	★★★★★ (Gold standard)	★★★☆☆ (Research only)	Reference; validation of other methods
DXA	★★★★☆	★★★★☆	Sarcopenia, osteoporosis, body fat%, VAT estimation; serial monitoring
CT / MRI	★★★★★ (tissue-specific)	★★★☆☆ (CT radiation)	VAT, IMAT, organ-specific, L3-SMI in oncology/surgery
Hydrostatic / Bod Pod	★★★★☆	★★★☆☆	Controlled clinical/sports settings; 2C model caveats
Isotope dilution	★★★★★ (TBW-specific)	★★★☆☆ (Lab required)	TBW and ECW/ICW in fluid research; renal disease
Multi-freq BIA	★★★☆☆	★★★★☆	Phase angle for nutritional risk; fluid monitoring in dialysis
Standard BIA	★★★☆☆ (population variation)	★★★★★	Screening; community settings; monitor trends in healthy populations
Anthropometry (WC, skinfolds)	★★★☆☆	★★★★★	Mass screening; CVD risk stratification; field nutrition assessment
Ultrasound (POCUS)	★★★☆☆	★★★★★ (bedside)	ICU/hospital muscle monitoring; rectus femoris in critical illness

V. DISEASE-ASSOCIATED BODY COMPOSITION ALTERATIONS

A. Obesity — Phenotypic Heterogeneity

Obesity (BMI ≥ 30) is characterized by excess fat mass but its health implications vary considerably based on fat distribution pattern:

Obesity Phenotype	Characteristics Metabolic Risk Assessment
Metabolically Healthy Obesity (MHO)	Excess fat but minimal VAT; normal metabolic profile (BP, lipids, glucose, insulin sensitivity). ~20–30% of obese individuals. NOT truly benign — most transition to metabolically unhealthy obesity over time. May require body composition (DXA/CT) to identify
Metabolically Unhealthy Obesity (MUO)	$\uparrow\uparrow$ VAT; $\uparrow$ liver fat; $\uparrow$ IMAT; insulin resistance; dyslipidaemia; hypertension. Dominant phenotype. Weight loss + exercise preferentially reduces VAT (responds better than SAT to caloric restriction and aerobic exercise)
Normal Weight Obesity (NWO)	Normal BMI but $\uparrow$ FM% ($>30\%$ women, $>25\%$ men) + low FFM. Often sedentary adults, older women. Undetected by BMI. Requires BIA or DXA. Associated with metabolic syndrome despite 'healthy' weight
Sarcopenic Obesity	Concurrent excess fat + low muscle mass. Double burden: metabolic risk from $\uparrow$ VAT + functional decline from $\downarrow$ muscle. Increasingly prevalent in elderly. Requires body composition assessment for diagnosis. Treatment: combined resistance training + protein-adequate hypocaloric diet

B. Cancer Cachexia

Cachexia is a multifactorial metabolic syndrome defined (Fearon et al., 2011) as: weight loss $>5\%$ over 6 months (or BMI <20 + weight loss $>2\%$ or sarcopenia + weight loss $>2\%$). Distinguished from simple starvation by the presence of ongoing inflammation and catabolism that is not fully reversible with nutritional support alone.

- Pathophysiology: Tumour-derived pro-inflammatory cytokines (IL-6, IL-1 β , TNF- α , IFN- γ) + tumour-specific factors (PIF — proteolysis-inducing factor, LMF — lipid mobilizing factor) $\rightarrow$ $\uparrow$ muscle protein catabolism (ubiquitin-proteasome system) + $\downarrow$ muscle protein synthesis ($\downarrow$ mTORC1 via AMPK, $\uparrow$ myostatin) + $\uparrow$ fat lipolysis $\rightarrow$ simultaneous loss of both FM and FFM, with muscle loss predominating
- Staging: Pre-cachexia (weight loss $\leq 5\%$, early metabolic changes) $\rightarrow$ Cachexia (weight loss $>5\%$ or criteria above) $\rightarrow$ Refractory cachexia (rapidly progressive disease, unresponsive to treatment, expected survival <3 months)
- Nutritional intervention: Cannot reverse cachexia alone but can attenuate muscle loss. High protein (1.2–2.0 g/kg/day); EPA/DHA omega-3 (attenuates NF- κ B, $\downarrow$ PIF signaling, preserves muscle; 2g EPA/day in studies); anti-inflammatory dietary pattern; resistance exercise when possible; appetite stimulants (megestrol acetate, mirtazapine); investigational: anti-myostatin antibodies, ghrelin agonists

C. Sarcopenic Obesity — A Growing Clinical Challenge

Sarcopenic obesity (SO) represents the co-existence of low muscle mass/function and excess adiposity. It is increasingly prevalent with population aging and physical inactivity, affecting an estimated 10–18% of older adults. Its pathogenesis involves mutual amplification of fat-driven inflammation and muscle-driven anabolic resistance:

- Mechanisms: $\uparrow$ VAT $\rightarrow$ $\uparrow$ IL-6, TNF- α , resistin $\rightarrow$ promote muscle catabolism + anabolic resistance; $\uparrow$ IMAT $\rightarrow$ $\uparrow$ local lipotoxicity $\rightarrow$ impairs myocyte insulin signaling; $\downarrow$ muscle mass $\rightarrow$ $\downarrow$ REE $\rightarrow$ positive energy balance $\rightarrow$ $\uparrow$ fat accumulation — a vicious cycle
- Diagnostic criteria (ESPEN/EASO Consensus 2022): Low muscle mass (ASMI by DXA or BIA) + low muscle function (grip strength or gait speed) + adiposity (BF% $\geq$ 30% women / $\geq$ 25% men; or WC $\geq$ 88cm women / $\geq$ 102cm men). Requires body composition assessment — BMI alone is insufficient
- Nutritional management: Hypocaloric diet (500–750 kcal/day deficit) but must be protein-adequate ($\geq$ 1.2–1.5 g/kg/day) to minimize muscle loss during fat reduction; leucine-rich protein (whey, eggs) at each meal to maximize MPS; omega-3 supplementation (2–3g EPA+DHA/day); combined with supervised progressive resistance training (the most critical co-intervention)

Assessment Pearl: *Phase angle (BIA) is particularly useful in sarcopenic obesity — BMI and even DXA fat% may appear normal while PhA reveals the metabolic risk of reduced cell membrane integrity and intracellular water. PhA < 5° in elderly signals significant nutritional risk regardless of other body composition metrics.*

D. Eating Disorders & Body Composition

- Anorexia nervosa (AN): Loss of FM and FFM in proportion; preservation of brain volume preferentially at expense of visceral and musculoskeletal mass; bone density severely compromised — dual oestrogen deficiency + malnutrition $\rightarrow$ irreversible BMD loss; cardiac muscle atrophy ($\downarrow$ left ventricular mass $\rightarrow$ bradycardia, arrhythmia risk); DXA monitoring essential; refeeding syndrome risk in severe malnutrition (BMI <15) — careful refeeding protocol mandatory
- Relative Energy Deficiency in Sport (RED-S): Insufficient energy availability (<30 kcal/kg FFM/day) in athletes $\rightarrow$ hormonal disruption ($\downarrow$ oestrogen/testosterone, $\downarrow$ IGF-1, $\uparrow$ cortisol) $\rightarrow$ impaired bone formation + $\uparrow$ stress fracture risk + $\downarrow$ muscle protein synthesis. The Female Athlete Triad (low energy availability, menstrual dysfunction, low BMD) is a subset of RED-S

VI. CLINICAL INTEGRATION — BODY COMPOSITION IN PRACTICE

A. Nutritional Assessment Framework Using Body Composition

Body composition assessment should be integrated into a comprehensive nutrition assessment alongside dietary intake, biochemical markers, clinical history, and functional assessment. The ABCDE framework:

ABCDE Component	Body Composition Integration
A — Anthropometrics	BMI (screening); WC (VAT proxy); WHR/WHtR (distribution risk); MUAC (muscle protein reserve); skinfold thickness (FM estimation); serial weight (trend over time)
B — Biochemical	Phase angle (BIA — cell membrane integrity); serum albumin/prealbumin (visceral protein status — but also negative acute phase reactants); creatinine-height index (muscle mass surrogate); urinary 3-methylhistidine (muscle protein catabolism); IGF-1 (anabolic status)
C — Clinical	Grip strength (primary sarcopenia criterion; strongest predictor of adverse outcomes across all ages); functional tests: gait speed, 6-Minute Walk Test, Short Physical Performance Battery (SPPB), Timed Up & Go (TUG); visual muscle wasting assessment (temporal wasting, thenar eminence)
D — Dietary	Energy adequacy relative to calculated needs (adjust for body composition — use FFM-based REE equations); protein quality and timing; calcium and vitamin D adequacy for bone; micronutrient status affecting muscle (Mg^{2+} , vitamin D, potassium) and bone (vitamins K2, C, magnesium)
E — Environmental/Functional	Physical activity assessment (PAL); sedentary time; functional independence; social determinants affecting nutrition access and preparation capacity

B. Energy Expenditure Estimation with Body Composition Data

Resting energy expenditure (REE) can be estimated more accurately using body composition data than standard weight-based or height-weight equations, particularly in extremes of weight, age, or disease:

Cunningham Equation (FFM-based REE — recommended for accuracy):

$$\text{REE (kcal/day)} = 500 + 22 \times \text{FFM (kg)}$$

Where FFM is from DXA or BIA. More accurate than Harris-Benedict in obese, athletes, and elderly

Mifflin-St Jeor Equation (weight-based — most validated in general use):

$$\text{Men: REE} = 10 \times W + 6.25 \times H - 5 \times \text{Age} + 5 \quad \text{Women: REE} = 10 \times W + 6.25 \times H - 5 \times \text{Age} - 161$$

W = weight in kg; H = height in cm. Most accurate weight-based equation for normal and obese adults.

Counseling Tip: In sarcopenic obese patients: use adjusted body weight (ABW) = IBW + 0.25 × (actual weight – IBW) or FFM-based equations to avoid over-estimating REE based on fat mass. Fat mass contributes relatively little to REE — REE is driven primarily by FFM (especially organ mass: liver, brain, kidneys, heart contribute ~60–70% of REE despite being <10% of body mass).

C. Serial Body Composition Monitoring — Practical Considerations

- Standardize conditions: Same time of day, hydration state, equipment, technician, and site for all serial measurements — even small protocol variations exceed the actual change being detected
- Understand precision error: DXA least significant change (LSC) for total body fat $\approx 1.0\text{--}1.5$ kg; for BMD $\approx 2\text{--}3\%$ — changes below LSC are not biologically meaningful; apply LSC when interpreting individual patient data
- Time frames for detectable change: Muscle protein synthesis responds within days but measurable DXA lean mass change requires 8–12 weeks of consistent training/nutrition; BMD changes require 6–12 months minimum (DEXA recommended annually for osteoporosis monitoring)
- Choose the right tool for the question: Fluid management in ICU $\rightarrow$ BIA phase angle + daily weight; Sarcopenia screening $\rightarrow$ DXA + grip strength; VAT assessment $\rightarrow$ waist circumference (field) or CT L3 (clinical/research); Bone health $\rightarrow$ DXA (DEXA) with T-score; Population screening $\rightarrow$ BMI + WC

Lecture 2 Summary — Key Principles

- Body composition models: 2C (FM + FFM) is foundational; 4C (FM + TBW + BMC + protein) is the gold standard; DXA provides a practical 3C approximation; CT/MRI provides tissue-specific quantification
- Lifespan arc: Peak muscle mass $\sim 25\text{--}30$ years $\rightarrow$ slow loss $0.5\text{--}1\%$ /year; peak BMD $\sim 25\text{--}30$ years $\rightarrow$ accelerated loss post-menopause ($2\text{--}5\%$ /year); fat mass redistributes to android/visceral pattern with age even without weight change
- Sarcopenia (EWGSOP2): Low grip strength + low ASMI by DXA; accelerated by inflammation, anabolic resistance, inactivity. First-line intervention: resistance training + $1.2\text{--}1.6$ g protein/kg/day with leucine-rich sources at each meal
- Measurement hierarchy: 4C > DXA/CT $\approx$ isotope dilution > Bod Pod > MF-BIA > skinfolds $\approx$ anthropometry; choose method based on clinical question, setting, and patient population
- BMI limitations: Cannot distinguish FM from FFM; misses normal weight obesity, sarcopenic obesity, and muscularity in athletes. Always combine with waist circumference and where possible BIA or DXA
- Phase angle (BIA): Reflects cell membrane integrity; low PhA ($<5^\circ$) is a powerful independent predictor of mortality across cancer, CKD, sepsis, and malnutrition — practical and underutilised clinical tool
- Cachexia: Distinct from starvation (ongoing inflammation prevents full reversal with nutrition alone); staging pre-cachexia $\rightarrow$ cachexia $\rightarrow$ refractory is critical for realistic goal-setting with patients and families

Suggested Reading:

1. Wells JCK & Fewtrell MS (2006) Measuring body composition. Arch Dis Child. |
2. Cruz-Jentoft AJ et al. (2019) Sarcopenia: revised European consensus on definition and diagnosis (EWGSOP2). Age Ageing. |
3. Duren DL et al. (2008) Body composition methods. J Diabetes Sci Technol. |
4. Fearon K et al. (2011) Definition and classification of cancer cachexia. Lancet Oncol. |
5. Bosy-Westphal A & Mueller MJ (2015) Measured and estimated resting energy expenditure. Curr Opin Clin Nutr Metab Care.

Module 3

Nutritional Assessment & Specialized Needs

Professor Osama Awad Salih

NUTRITION & DIETETICS

MS Program Lecture Series

Nutritional Assessment & Specialized Needs

Evaluating Intake & Requirements Across Populations

Assessment Trends • Life Cycle Nutrition • Food Safety & Adequacy

Professor Osama Awad Salih

Lecture 1

Assessment Trends

Dietary assessment steps
In-vitro & in-vivo methods

Lecture 2

Life Cycle Nutrition

Pregnancy • Lactation • Infancy
Adults • Elderly • Vegetarians

Lecture 3

Food Safety & Adequacy

Food chain • Processing •
Storage
Toxicants • Allergens

LECTURE 1

Assessment Trends

Three Steps of Dietary Assessment & Nutritional Evaluation of Elements (In-Vitro & In-Vivo)

Nutrition & Dietetics MS Program | Nutritional Assessment & Specialized Needs Series

Learning Outcomes — By the end of this lecture, students will be able to:

- Describe the three sequential steps of nutritional assessment and the purpose of each step
- Compare and critically evaluate dietary assessment methods: 24-hour recall, food frequency questionnaire, food record, and dietary history
- Apply population-level dietary survey methodologies and understand their limitations for individual assessment
- Explain the principles of in-vitro nutritional evaluation: chemical analysis, bioavailability assays, and digestion models
- Describe in-vivo methods: balance studies, isotope tracer techniques, biomarker-based assessments, and clinical biomarkers
- Integrate multiple lines of assessment evidence for a complete nutritional evaluation of an individual or population

I. THE THREE STEPS OF NUTRITIONAL ASSESSMENT

A. Step 1 — Dietary Intake Assessment (What Goes In)

The first step quantifies actual food and nutrient consumption. It forms the foundation of nutritional evaluation but is invariably limited by reporting bias, day-to-day variability, and the complexity of translating foods into nutrients. No single method is perfect, selection depends on study design, population, resources, and purpose.

Overview of Dietary Assessment Methods — Comparative Framework

Method	Time Frame	Strengths	Limitations
24-Hour Dietary Recall (24HR)	Previous 24 hours	Relatively unbiased; does not alter eating behaviour; low respondent burden; can be administered by interview; suitable for illiterate populations	Single day does not represent usual intake (high day-to-day variability); relies on memory; portion size estimation errors; interviewer skill-dependent
Food Frequency Questionnaire (FFQ)	Past month/year (habitual intake)	Captures usual diet; low cost; self-administered; suitable for large epidemiological studies; can assess specific food groups or nutrients	Cannot capture precise amounts; food list may miss culturally specific foods; respondent recalls are reconstructed (cognitive distortion); validated only for specific populations
Food/Diet Record (Diary)	3–7 days prospective	Most accurate for actual intake; no reliance on memory; can capture	Reactive — recording changes eating behaviour (Hawthorne)

		detailed amounts and brands; can use weighed food records for highest precision	effect); high respondent burden → under-reporting of high-fat/sugar foods; labour-intensive analysis; not suitable for large studies
Dietary History (Diet History)	Habitual/usual intake (1+ months)	Comprehensive — combines 24HR + food record + cross-check interview; captures meal patterns and context; excellent for clinical settings	Very time-intensive (45–90 min); requires skilled interviewer; retrospective; limited validation data

1. The Multiple-Pass 24-Hour Recall (USDA Automated Self-Administered 24HR — ASA24)

The gold standard individual dietary recall method employs the USDA 5-step multiple-pass method (MPM) to maximize completeness and minimize omissions:

- Pass 1 — Quick List: Respondent rapidly lists all foods/beverages consumed without interruption
- Pass 2 — Forgotten Foods Probe: Interviewer probes for commonly forgotten categories (beverages, condiments, snacks, supplements, alcohol)
- Pass 3 — Time & Occasion: Establishes timing and eating occasion for each item (meal context)
- Pass 4 — Detail Cycle: For each item — detailed description (brand, preparation method), amount (using portion size models, food photographs, standard containers), and additions (sauces, fats, seasonings)
- Pass 5 — Final Probe: Final check for anything forgotten since the Quick List

Assessment Pearl: *Minimum two non-consecutive 24HRs (ideally 3–4 including a weekend day) are required to estimate usual intake distributions using statistical modelling approaches (NCI MSMD method, SPADE). One day captures only the 'observed' intake, not usual intake — a critical distinction for nutritional epidemiology.*

2. Food Frequency Questionnaire (FFQ) — Design & Validation

FFQs list foods/food groups (typically 100–150 items) with frequency response categories (never to 6+ times/day) and optional portion size questions. Nutrient intake is calculated by multiplying frequency × portion × nutrient composition per serving (using composition databases: USDA FoodData Central, UK Nutrient Databank, regional databases).

- Semi-quantitative FFQ (Block, Willett/Harvard): Specify a standard portion; respondent estimates frequency relative to that portion
- Quantitative FFQ: Respondent specifies both frequency and portion size — more accurate but greater burden
- Validation: FFQs must be validated against criterion methods (multiple 24HRs or biomarkers) in the target population before use. Pearson or Spearman correlation typically 0.5–0.7 for most nutrients vs. multiple 24HR criterion. Validation is population-specific — a US-validated FFQ is not valid for use in Egyptian or Korean populations without recalibration
- DHQ-III (Diet History Questionnaire III): USDA/NCI web-based FFQ; 134 food items; validated for US adults; free access

3. Dietary Assessment Technology — Emerging Approaches

- Mobile/smartphone apps: MyFitnessPal, Cronometer, Nutritics — barcode scanning, photo logging, voice input; improve engagement but introduce selection bias; accuracy depends on food database completeness

- Image-assisted dietary assessment: FDDb (Food Database & Diary), GoCARB — machine learning object recognition from food photos → automated portion estimation; promising but still requires validation for clinical use
- Wearable sensors: BITE'R (Jaw motion sensor), wrist accelerometers (chewing detection) — eating occasions and meal duration; not yet validated for nutrient intake
- Metabolomics-based dietary assessment: Plasma/urine metabolite profiles as objective biomarkers of dietary patterns (nutritional metabolomics); correlate with self-reported intakes but require large reference databases; future of objective dietary assessment

B. Step 2 — Nutritional Status Evaluation (What the Body Shows)

The second step evaluates whether dietary intake has translated to adequate nutritional status within the body. This encompasses anthropometric, biochemical, clinical, and dietary (ABCD) assessment — moving from intake to outcome.

Anthropometric Assessment

See Lecture 2 of Body Regulation & Composition series for detailed coverage. Key tools: BMI, waist circumference, skinfold thickness, MUAC, DXA. In nutritional assessment context:

- Paediatric anthropometry: Weight-for-age (WAZ), Height-for-age (HAZ — stunting: HAZ < -2 SD), Weight-for-height (WHZ — wasting: WHZ < -2 SD; severe acute malnutrition: WHZ < -3 or MUAC < 115 mm), BMI-for-age z-scores. WHO and CDC 2000 growth charts are international references
- Global Malnutrition Screening: Mini Nutritional Assessment (MNA) for elderly; Malnutrition Universal Screening Tool (MUST) for adults; Subjective Global Assessment (SGA) — clinician rates A/B/C based on history, GI symptoms, functional status, physical exam findings

Biochemical Assessment — Key Biomarkers

Biomarker Category	Key Markers	Interpretation & Limitations
Protein/nitrogen status	Serum albumin ($t_{1/2}$ ~20 days), prealbumin/transthyretin ($t_{1/2}$ ~2 days), retinol-binding protein ($t_{1/2}$ ~12h), transferrin ($t_{1/2}$ ~8 days), urinary creatinine-height index	All are negative acute-phase reactants — levels fall with inflammation/stress independent of nutritional status. Prealbumin better short-term marker. NOT reliable for acute malnutrition diagnosis in inflammatory states. CRP must be measured concurrently to interpret
Iron status	Serum ferritin (storage — elevated in inflammation), serum iron, TIBC, transferrin saturation, soluble transferrin receptor (sTfR), hepcidin, reticulocyte haemoglobin content (CHr), zinc protoporphyrin	Ferritin is positive acute-phase reactant — elevated despite iron deficiency in inflammation. sTfR/log ferritin ratio (Thomas plot) distinguishes iron deficiency from anaemia of inflammation. CHr reflects iron supply to erythropoiesis in real-time
Vitamin status	25(OH)D (vitamin D); serum retinol (vitamin A — insensitive until stores depleted); RBC transketolase activation (thiamin); erythrocyte glutathione reductase activation (riboflavin); plasma PLP (B6); serum/RBC	Functional markers (MMA for B12, EGRAC for B2, TPPE for B1) detect deficiency before serum levels fall. Always pair serum B12 with MMA and homocysteine — serum B12 can be normal with functional deficiency. 25(OH)D is definitive for vitamin D status

	folate; serum B12 + functional markers (MMA, homocysteine); serum ascorbate (vitamin C)	
Mineral status	Serum Zn (poor marker — affected by inflammation, albumin, time of day), RBC Zn (better chronic marker), plasma Cu, serum Se (Selenoprotein P preferred), serum Mg (poor for intracellular stores — use RBC Mg or retention test), serum/urine iodine (spot urine iodine: UIC — population indicator)	No reliable serum marker for Zn or Mg intracellular stores. Urine iodine concentration (UIC) is a population-level indicator only — not for individual diagnosis. Serum Se poorly reflects tissue stores; Selenoprotein P is superior
Oxidative stress / inflammation	CRP (hs-CRP for subclinical), IL-6, TNF- α , fibrinogen (identify confounded results); F2-isoprostanes (oxidative stress gold standard); 8-OHdG (DNA oxidation); MDA/TBARS (lipid peroxidation); phase angle (BIA — cell integrity)	Always measure CRP alongside nutritional biomarkers to interpret in inflammatory states. CONUT score (CRP + albumin + cholesterol + lymphocytes) integrates inflammation and nutrition for hospitalized patient risk stratification

Clinical Note: *The GLIM Criteria (Global Leadership Initiative on Malnutrition, 2019): International consensus criteria for malnutrition diagnosis requiring at least 1 phenotypic criterion (weight loss >5%/6 months or >10%/>6 months; low BMI <20 kg/m² if <70 years, <22 if ≥70 years; reduced muscle mass) AND at least 1 etiologic criterion (reduced food intake/assimilation; disease burden/inflammation). The GLIM framework replaces older SGA-based definitions and standardizes malnutrition diagnosis globally.*

C. Step 3 — Integration & Diagnosis (Connecting Intake to Status to Outcomes)

The third step synthesizes all data streams — dietary intake, biomarkers, anthropometrics, clinical signs, and functional status — into a diagnostic conclusion and intervention plan. This includes DRI comparison, pattern recognition of deficiency syndromes, causal attribution, and prioritization of clinical interventions.

- DRI framework application: Compare intake to Estimated Average Requirement (EAR) using probability approach; if EAR not available, compare to Adequate Intake (AI). Population intakes below EAR indicate risk of inadequacy; intakes above Tolerable Upper Intake Level (UL) indicate risk of adverse effects. For individuals: single-day intake below EAR does not diagnose deficiency — must average multiple days and confirm with biomarkers
- Nutrient-symptom correlation: Match clinical signs to deficiency syndromes (e.g., angular cheilitis + glossitis + normochromic anaemia → riboflavin; perifollicular haemorrhage + bleeding gums → vitamin C; dermatitis + diarrhoea + dementia → niacin)
- Causal pathway analysis: Rule out non-dietary causes of deficiency (malabsorption, increased losses, genetic errors of metabolism, drug-nutrient interactions) before attributing to low intake alone
- Monitoring framework: Establish pre-intervention baseline → define measurable outcome targets → set reassessment timeline (biomarker-specific: prealbumin weekly; 25(OH)D quarterly; BMD annually)

II. NUTRITIONAL EVALUATION OF ELEMENTS — IN-VITRO METHODS

A. Principles of In-Vitro Nutritional Analysis

In-vitro (Latin: 'in glass') methods evaluate nutrients outside a living organism — in laboratory conditions. They range from direct chemical analysis of food composition to sophisticated simulated digestion models that predict bioavailability. In-vitro methods are faster, cheaper, and more controllable than in-vivo studies, but have inherent limitations in translating to physiological reality.

B. Food Composition Analysis Methods

Analytical Method	Principle, Applications & Limitations
Proximate Analysis (Weende System)	Separates food into: moisture (gravimetric drying), crude protein (Kjeldahl/Dumas nitrogen $\times 6.25$), crude fat (Soxhlet ether extraction), crude fibre (acid-alkaline digestion residue), ash (muffle furnace 550°C), nitrogen-free extract ($\text{NFE} = 100 - \text{sum}$). Standard regulatory and food labelling basis. Limitations: crude fibre underestimates dietary fibre; NFE is calculated not measured; protein conversion factor 6.25 assumes uniform N content (not accurate for all foods)
AOAC Dietary Fibre Methods	Total Dietary Fibre (TDF) by enzymatic-gravimetric method (AOAC 985.29/991.43); separates soluble (SDF) and insoluble (IDF); newer AOAC 2011.25 captures low-molecular-weight soluble fibre (resistant dextrin, fructooligosaccharides) missed by older methods. Critical because fermentability and physiological effects differ between fractions
Amino Acid Analysis	Acid hydrolysis (6N HCl , 110°C , 24h) $\rightarrow$ ion-exchange chromatography or reverse-phase HPLC with ninhydrin/fluorescence detection. Provides complete amino acid profile. Limitation: tryptophan destroyed by acid hydrolysis (requires alkaline hydrolysis separately); methionine and cysteine oxidized unless protected. Used for PDCAAS and DIAAS calculation
Lipid Analysis & Fatty Acid Profiling	Total fat: Soxhlet extraction (total lipid); Folch or Bligh-Dyer methods (polar lipids). Fatty acid profile: saponification + methylation $\rightarrow$ fatty acid methyl esters (FAMES) $\rightarrow$ gas chromatography (GC-FID). Identifies saturated, monounsaturated, polyunsaturated, trans fatty acid content. Required for omega-3 EPA/DHA quantification in foods and supplements
Mineral/Trace Element Analysis	Dry or wet ashing $\rightarrow$ acid dissolution $\rightarrow$ Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), or ICP-Optical Emission Spectrometry (ICP-OES). ICP-MS is gold standard — simultaneous multi-element analysis at ppb-ppt levels. Used for mineral content of foods and biological samples (blood, urine, hair, nails)
Vitamin Analysis	Water-soluble vitamins: HPLC with UV/fluorescence/electrochemical detection (vitamin C, B vitamins); microbiological assays (folate — <i>Lactobacillus rhamnosus</i> ; B12 — <i>Lactobacillus delbrueckii</i>). Fat-soluble vitamins: saponification + solvent extraction $\rightarrow$ HPLC (vitamins A, D, E, K). LC-MS/MS increasingly used for simultaneous multi-vitamin profiling. Each vitamin requires validated extraction protecting against degradation (light, heat, oxidation)
Phytochemical Analysis	Total phenolics: Folin-Ciocalteu colorimetric (total gallic acid equivalents — non-specific but widely used). Total flavonoids: AlCl_3 colorimetric.

Specific polyphenols: HPLC-DAD or LC-MS/MS (quantitative identification). Carotenoids: saponification + HPLC-DAD or LC-MS. Glucosinolates: sinigrin equivalent by HPLC. Antioxidant capacity assays: ORAC, DPPH, FRAP, ABTS (different mechanisms — results not interchangeable). All assays have matrix-specific validity issues

C. Bioavailability Assessment — In-Vitro Models

Food composition data tells us what is present in food — bioavailability assessment tells us what fraction is actually absorbed and utilized. In-vitro bioavailability models simulate digestive conditions to predict the bio accessible fraction (the fraction released from the food matrix and potentially available for absorption).

1. In-Vitro Digestion Models

Model	Description & Application
Static in-vitro digestion (INFOGEST Harmonized Protocol, 2019)	Three-phase sequential digestion: (1) Oral phase: salivary amylase + α -amylase, 2 min, pH 7.0; (2) Gastric phase: pepsin, pH 3.0, 2 hours, 37°C, agitation; (3) Small intestinal phase: pancreatin (pancreatic enzymes) + bile salts, pH 7.0, 2 hours. Dialysis or ultracentrifugation separates soluble (bioaccessible) fraction. Most widely used standardized protocol for food bioavailability research
Dynamic in-vitro digestion (TNO TIM, DIDGI, SHIME)	Computer-controlled models simulating dynamic gastric emptying, peristaltic contractions, changing pH, and sequential enzyme additions. TIM-1 (TNO Intestinal Model) simulates small intestine; TIM-2 simulates colon (includes microbiota). More physiologically relevant but expensive and complex. SHIME (Simulator of the Human Intestinal Microbial Ecosystem) includes a defined human microbiota — useful for prebiotic and polyphenol fermentation studies
Caco-2 Cell Monolayer Model	Caco-2 cells (human colorectal adenocarcinoma) differentiate into enterocyte-like cells with brush border enzymes and tight junctions. Apply bioaccessible fraction from in-vitro digestion to apical surface → measure transport to basolateral compartment → bioavailable fraction estimate. Gold standard for mineral (Fe, Zn, Ca) intestinal absorption studies. Limitations: no mucus layer, no peristalsis, no immune cells, no microbiota
Equilibrium Dialysis & Ultrafiltration	Dialysis membrane (molecular weight cut-off 10–14 kDa) placed in digestion vessel; small soluble molecules (minerals, vitamins) diffuse across; dialysate = bioaccessible fraction. Simpler than Caco-2 but no cellular transport information. Widely used in mineral bioavailability research (dialyzable iron method for non-haeme iron)
Simulated Salivary/Gastric Fluid (SGF/SIF)	USP-defined simulated gastric fluid (pH 1.2, pepsin) and simulated intestinal fluid (pH 6.8, pancreatin) for pharmaceutical dissolution testing — adapted for food applications. Used to evaluate phytochemical stability during digestion

2. Protein Quality Assessment Methods

Method	Calculation & Current Status
PDCAAS (Protein Digestibility-Corrected Amino Acid Score)	PDCAAS = (mg limiting amino acid per g protein / mg same AA in reference pattern) × True Faecal Digestibility. Reference: FAO 1991 pre-school pattern. Truncated at 1.0. Standard for food labelling (FDA, Codex Alimentarius) until DIAAS adoption. Limitation: faecal digestibility overestimates true ileal digestibility (bacterial AA synthesis in colon inflates value)
DIAAS (Digestible Indispensable Amino Acid Score — FAO 2013)	DIAAS = (mg digestible dietary indispensable AA in 1g dietary protein / mg same AA in reference protein) × 100. Uses True Ileal Digestibility (TID) of each individual amino acid measured at terminal ileum (pig or rat model; in-vitro INFOGEST proxy validated). Not truncated at 100 — allows differentiation of exceptional proteins. Three age-specific reference patterns (infant <6m; child 0.5–3y; older child/adult). DIAAS > 100: complete high-quality protein; DIAAS 75–100: adequate protein; < 75: limited (requires complementation). Whey > egg > soy > wheat (DIAAS values ~1.09, 1.0, 0.91, 0.40 respectively)
Nitrogen Balance Study (In-Vivo reference for protein requirements)	N intake – N output (urine + faeces + integumental + miscellaneous losses). Positive balance = anabolism; negative = catabolism. Used to establish protein requirements (EAR). Technically demanding — requires complete urine and faecal collection, low-N diet control periods. Replaced partly by indicator amino acid oxidation (IAAO) technique for determining protein requirements
Indicator Amino Acid Oxidation (IAAO)	Fed graded protein intakes; ¹³ C-phenylalanine tracer given; measure ¹³ CO ₂ in breath. At inadequate protein intake, protein synthesis is limited → excess phenylalanine oxidized (high ¹³ CO ₂ excretion). As protein intake increases to requirement → phenylalanine incorporated into protein → ¹³ CO ₂ decreases → breakpoint = protein requirement. Non-invasive; suitable for vulnerable populations (infants, elderly, pregnant women) where N balance is challenging

III. NUTRITIONAL EVALUATION OF ELEMENTS — IN-VIVO METHODS

A. Principles of In-Vivo Assessment

In-vivo (Latin: 'within the living') methods evaluate nutrient metabolism, status, and requirements in living organisms — humans or animal models. They provide physiological validity that in-vitro methods cannot replicate but are more complex, expensive, time-consuming, and ethically constrained.

B. Stable Isotope Tracer Techniques

Stable isotopes are non-radioactive, naturally occurring variants of elements differing in neutron number (e.g., ¹³C, ²H, ¹⁵N, ¹⁸O, ⁵⁸Fe, ⁶⁵Zn, ⁷⁰Se). They can be incorporated into nutrients and tracked in biological fluids without radiation risk — suitable for use in pregnant women, infants, and children.

Application	Isotope & Protocol	Information Obtained
Iron absorption	^{57}Fe or ^{58}Fe in test meal → blood sampling at 14 days → ICP-MS measurement of erythrocyte isotope enrichment; two-isotope method (reference + test meal simultaneously)	Absolute iron absorption (% of dose); comparison of test vs reference meal; effect of enhancers/inhibitors (vitamin C, phytate, polyphenols)
Zinc absorption	^{67}Zn or ^{68}Zn oral dose + ^{70}Zn IV reference → urine collection → ICP-MS	True zinc absorption (Zn oral dose/Zn IV reference); zinc homeostasis; effect of phytate
Calcium absorption	^{42}Ca or ^{44}Ca oral + ^{43}Ca IV reference → urine ICP-MS; or ^{44}Ca dual isotope technique	Fractional calcium absorption; effect of vitamin D, lactose, oxalate
Protein/amino acid kinetics	^{13}C -leucine, ^{15}N -glycine tracers → breath $^{13}\text{CO}_2$ + urine ^{15}N → whole-body protein turnover, synthesis, breakdown rates	Protein synthesis rate, fractional synthesis rate (FSR) of muscle; effect of feeding, exercise, disease on protein kinetics
Vitamin D metabolism	$^2\text{H}_3$ -vitamin D ₃ oral dose → serum sampling → LC-MS/MS tracking of deuterated 25(OH)D ₃ formation	Vitamin D absorption and hepatic 25-hydroxylation efficiency; vitamin D bioavailability from different food sources
Total body water / energy expenditure	Doubly Labelled Water (DLW): $^2\text{H}_2^{18}\text{O}$ administered → rate of ^2H elimination (water output) vs ^{18}O elimination (water + CO_2) → total CO_2 production → total energy expenditure (TEE) over 1–3 weeks	Gold standard for free-living TEE; physical activity level (PAL = TEE/REE); suitable for all age groups; 2-week window

C. Balance Studies

Balance studies quantify net retention of a nutrient by measuring intake minus all measurable losses (urine, faeces, sweat, integumental losses). Nitrogen balance is the classic example but balance studies exist for calcium, zinc, sodium, and other minerals.

- Metabolic ward conditions: Controlled food intake (weighed, prepared diet), complete collections of all excreta (24h urine × 7–14 days, complete faecal collection with marker), standardised activity. Extremely labour-intensive and expensive — used primarily to define dietary requirements and study nutrient-nutrient interactions
- Limitations: Does not account for changes in body composition distribution; positive nitrogen balance includes non-muscle proteins (albumin, immunoglobulins); complete collection of sweat and integumental losses is practically difficult; short study duration may miss adaptive responses
- Requirement determination: Nutrient requirement = amount needed to maintain zero balance (for minerals) or neutral to slightly positive balance (for nitrogen). EAR derived from balance study median requirement + factorial modelling

D. Biomarker-Based In-Vivo Assessment

Biomarkers provide objective, quantifiable measures of nutritional exposure, metabolism, or status in biological specimens — overcoming the limitations of self-reported dietary data.

Biomarker Type	Examples & Application
Nutrient concentration markers (Direct status markers)	Serum 25(OH)D (vitamin D); plasma retinol (vitamin A — insensitive until stores near depletion); plasma ascorbate (vitamin C — reflects recent intake); serum/plasma B12; erythrocyte folate (reflects tissue stores better than serum folate); plasma carotenoids (lycopene, lutein, β -carotene — reflect fruit/vegetable intake); plasma EPA/DHA % (reflects omega-3 status — Omega-3 Index)
Functional/metabolic markers (Reflect sufficiency for metabolic function)	Plasma MMA (methylmalonic acid — elevated in B12 deficiency even with normal serum B12); plasma homocysteine (elevated in folate, B12, B6 deficiency, MTHFR); EGRAC (erythrocyte glutathione reductase activation coefficient — riboflavin status); TPPE (transketolase pyrophosphate effect — thiamin status); plasma PTH (elevated in vitamin D insufficiency before 25(OH)D falls below 50 nmol/L); reticulocyte haemoglobin content (iron supply to erythropoiesis in real-time)
Recovery/excretion biomarkers (Reflect intake of food components)	Urinary nitrogen excretion (protein intake proxy — Bingham/Cade formula); urinary potassium (24h urine potassium $\approx$ dietary potassium); urinary sodium (24h collection = gold standard for sodium intake assessment — used in population surveys); urinary sucrose + fructose (added sugar intake proxy); urinary alkylresorcinols (whole grain wheat/rye intake — valid and objective biomarker); urinary isoflavones (soy isoflavone intake)
Concentration-response biomarkers (Population-level intake validation)	Plasma carotenoids validate fruit/vegetable intake in FFQ validation studies; urinary iodine concentration (UIC) for population iodine status (median UIC 100–199 μ g/L = adequate); hair/nail selenium (reflects 3-month status); hair zinc (chronic status — but affected by growth rate, treatments)
Omics-based nutritional biomarkers (Emerging field: nutritional metabolomics)	Untargeted plasma/urine metabolomics (1 H-NMR, LC-MS/MS) identifies hundreds of metabolites correlated with dietary patterns; specific dietary biomarker panels (FOODBALL project, PREDIMED-Plus); can objectively reconstruct dietary patterns from metabolome; reduces reliance on self-report; increasingly used in large cohorts (UK Biobank, NHANES)

E. Clinical In-Vivo Assessment Tools

Tool	Application & Key Details
DXA (Body Composition)	Fat mass, lean mass, bone mineral density. Gold standard for bone mineral content assessment. Sarcopenia criteria (EWGSOP2). Covered in detail in Body Composition lecture
Indirect Calorimetry	Measures O_2 consumption and CO_2 production $\rightarrow$ REE via Weir equation. Gold standard for energy expenditure in clinical nutrition. Respiratory Quotient ($RQ = VCO_2/VO_2$): fat oxidation RQ 0.7, carbohydrate 1.0, protein 0.8, lipogenesis >1.0 . Used in ICU, ventilated patients, post-surgical, obese patients where predictive equations are inaccurate. Metabolic carts increasingly used in clinical dietetics

Doubly Labelled Water (DLW)	Free-living total energy expenditure over 10–14 days. Reference method for validating physical activity questionnaires and dietary assessment methods. Used in requirement studies for infants, pregnant women, elderly
Grip Strength Dynamometry	Validated functional marker of muscle strength; primary sarcopenia criterion (EWGSOP2); strong predictor of all-cause mortality, hospitalisation, and complications across all clinical populations
Phase Angle (BIA)	Reflects cell membrane integrity and intracellular hydration. Low PhA (<5°) predicts poor nutritional status and mortality. Complements body composition assessment in inflammatory states where DXA is less interpretable
D ₃ -creatine (D ₃ Cr) dilution	Novel method for total body skeletal muscle mass measurement: oral D ₃ -creatine → enriches creatine/creatinine pool proportional to muscle mass → urine D ₃ -creatinine enrichment at steady state → total body muscle mass. More accurate than DXA lean mass (excludes connective tissue, skin). Emerging research method

Lecture 1 Summary — Key Principles

- Three steps of nutritional assessment: (1) Dietary intake assessment [24HR, FFQ, food record, dietary history] → (2) Nutritional status evaluation [anthropometry, biochemistry, clinical signs — ABCD framework] → (3) Integration & diagnosis [DRI comparison, biomarker synthesis, causal attribution, monitoring plan]
- 24HR Multiple-Pass Method (5 steps) is the gold standard individual recall; minimum 2 non-consecutive recalls required for usual intake estimation; FFQs capture habitual diet for epidemiology but require population-specific validation
- In-vitro methods: Proximate analysis, HPLC, ICP-MS for food composition; INFOGEST harmonised protocol for bioaccessibility; Caco-2 model for mineral transport; DIAAS (ileal digestibility) is the current gold standard for protein quality (superseding PDCAAS)
- In-vivo methods: Stable isotope tracers (⁵⁸Fe, ⁷⁰Se, D₃-water) for absorption and kinetics; balance studies for requirement determination; biomarkers stratified by type (status, functional, recovery, omics)
- Functional markers (MMA for B12, EGRAC for B2, TPPE for B1, IAAO for protein requirements) detect deficiency before serum concentrations fall — essential for sensitive nutritional evaluation
- GLIM Criteria (2019): Requires ≥1 phenotypic criterion (weight loss, low BMI, reduced muscle mass) + ≥1 etiologic criterion (reduced intake/assimilation or disease/inflammation) — international gold standard for malnutrition diagnosis

Suggested Reading:

1. Gibson RS (2005). Principles of Nutritional Assessment, 2nd Ed. |
2. INFOGEST harmonized static in vitro digestion method (Brodkorb et al., 2019). Nat Protoc. |
3. FAO (2013). Dietary Protein Quality Evaluation in Human Nutrition. |
4. Jenab M et al. (2009). Biomarkers in nutritional epidemiology. Br J Nutr.

LECTURE 2

Life Cycle Nutrition

Requirements for Pregnancy, Lactation, Infancy, Adulthood, the Elderly & Vegetarians

Nutrition & Dietetics MS Program | Nutritional Assessment & Specialized Needs Series

Learning Outcomes — By the end of this lecture, students will be able to:

- Describe the physiological adaptations of pregnancy and lactation that alter nutrient requirements
- Specify critical nutrient needs across each life stage with evidence-based DRI values and clinical rationale
- Identify high-risk nutritional deficiencies for each life stage and describe their consequences
- Design an evidence-based dietary guidance plan for pregnant women, lactating mothers, infants, adults, and elderly individuals
- Evaluate the nutritional adequacy of vegetarian and vegan dietary patterns and identify key nutrients requiring attention
- Apply nutrient interaction principles and life-stage-specific physiological changes to individualised dietary counselling

I. PREGNANCY NUTRITION

A. Physiological Adaptations in Pregnancy

Pregnancy induces profound physiological changes that alter nutrient absorption, metabolism, distribution, and requirements. These adaptations begin at conception and evolve throughout the three trimesters:

- Plasma volume expansion: ~45–50% increase by week 32 (haemodilution) → physiological anaemia of pregnancy (↓ haematocrit despite ↑ red cell mass); alters interpretation of serum nutrient concentrations
- GI adaptations: ↓ gastric motility (progesterone → ↑ transit time → ↑ absorption of minerals Fe, Ca); ↑ active transport of many nutrients (Ca²⁺ TRPV6 expression doubles; folate absorption ↑; Fe absorption triples in third trimester)
- Renal changes: GFR ↑ 50% → ↑ urinary losses of water-soluble vitamins, amino acids, glucose (glycosuria is normal), iodine
- Metabolic adaptation: First half — anabolic (fat deposition, glycogen storage); Second half — catabolic (↑ lipolysis, insulin resistance → ↑ glucose availability for foetus); ↑ basal metabolic rate ~15–20% by third trimester
- Placental transfer: Active and facilitated transport of glucose (GLUT1/3), amino acids (System A, L transporters), fatty acids (FATP transporters); passive diffusion of lipid-soluble vitamins; placenta prioritizes foetal needs — 'foetal parasitism' for most nutrients

B. Critical Nutrient Requirements in Pregnancy

Nutrient	Non-Pregnant	Pregnant	Key Rationale & Consequences of Deficiency
Folate (DFE)	400 µg/day	600 µg/day (+200 µg supplement recommended)	Neural tube closure: days 21–28 post-conception (before pregnancy confirmed). Deficiency → anencephaly, spina bifida (NTD). 70% reduction with periconceptional supplementation. MTHFR C677T carriers need additional methylfolate
Iron	18 mg/day (pre-menop.)	27 mg/day (+9 mg)	Foetal iron stores (primacy in 3rd trimester), expanded maternal RBC mass, blood loss at delivery. IDA → ↑ preterm birth, LBW, postpartum haemorrhage, impaired neurodevelopment. Screen at booking + 28 weeks (FBC, ferritin)
Iodine	150 µg/day	220 µg/day (+70 µg)	Foetal thyroid hormone synthesis (neurodevelopment). Deficiency → cretinism, intellectual disability, stillbirth, congenital hypothyroidism. Most critical nutrient for brain development. WHO: universal salt iodization + supplement in pregnancy
Calcium	1,000 mg/day	1,000 mg/day (same)	Foetal skeletal mineralization (~30 g Ca transferred to foetus). If dietary Ca inadequate → maternal bone resorption (PTHrP from mammary/placenta). AI maintained by ↑ intestinal absorption (1,25(OH) ₂ D doubles in pregnancy)
Vitamin D	600 IU/day	600 IU/day (many guidelines 1,000–2,000 IU)	Foetal bone development, immune programming (↓ asthma risk), pre-eclampsia risk reduction. 25(OH)D < 50 nmol/L prevalent in pregnancy globally. RCOG/ADA recommend 1,000 IU/day supplement for all pregnant women
DHA (omega-3)	AI 1.1 g ALA/day	~200–300 mg DHA/day (no formal DRI)	Foetal brain and retinal development (80% of brain DHA accumulated 3rd trimester–2 years). Seafood 2–3 ×/week or algal DHA supplement. Avoid high-mercury fish (shark, swordfish, king mackerel, tilefish)
Protein	46 g/day	71 g/day (+25 g)	Foetal tissue accretion, placenta, amniotic fluid, expanded maternal blood volume and uterus. Requirement highest in 3rd trimester (~1.1 g/kg/day). IAAO studies suggest actual need may be higher (1.2–1.5 g/kg/day)
Choline	425 mg/day	450 mg/day	Brain development (phosphatidylcholine in membranes, acetylcholine synthesis, methyl donor). Major dietary sources: eggs, meat, fish, liver. Deficiency → impaired hippocampal development, ↓ memory function. Most prenatal vitamins do not contain adequate choline

Gestational Diabetes Mellitus (GDM) — Nutritional Management

Clinical Note: GDM affects 7–14% of pregnancies globally (WHO diagnostic criteria: fasting glucose ≥ 5.1 mmol/L or 1h ≥ 10.0 or 2h ≥ 8.5 on 75g OGTT at 24–28 weeks). Nutritional management: consistent carbohydrate distribution (45–50% of energy, low GI sources preferred); avoid large carbohydrate loads; monitor postprandial glucose targets (1h < 7.8 mmol/L). Inadequate control → macrosomia, shoulder dystocia, neonatal hypoglycaemia, ↑ child obesity/T2DM risk. Myo-inositol supplementation (4g/day) emerging evidence for GDM prevention in high-risk women.

Weight Gain Guidelines in Pregnancy (IOM 2009):

Pre-pregnancy BMI	Recommended Total Gain	Rate in 2nd–3rd Trimester
Underweight (< 18.5)	12.5–18 kg	0.51 kg/week
Normal weight (18.5–24.9)	11.5–16 kg	0.42 kg/week
Overweight (25.0–29.9)	7–11.5 kg	0.28 kg/week
Obese (≥ 30.0)	5–9 kg	0.22 kg/week

II. LACTATION NUTRITION

A. Physiology of Lactation

Lactation is the most nutritionally demanding physiological state across the lifespan — energy and nutrient demands exceed those of pregnancy for most nutrients:

- Milk production: ~780 mL/day (first 6 months exclusively breastfed); ~600 mL/day when complementary foods introduced. Peak production ~1,200 mL/day in some women. Energy content: ~67 kcal/100 mL (mature milk)
- Hormonal control: Prolactin (anterior pituitary) → milk synthesis; Oxytocin (posterior pituitary) → milk ejection (let-down reflex — released by infant suckling, also by sight/sound/thought of infant; inhibited by stress/pain). Frequent feeding/pumping maintains prolactin levels — critical for establishing supply
- Milk composition stages: Colostrum (days 1–5): low volume, very high immunoglobulins (especially IgA), lactoferrin, growth factors, leukocytes — critical immunological priming; Transitional milk (days 5–14); Mature milk (>14 days): stable macronutrient composition but micronutrient content highly responsive to maternal diet

B. Nutrient Requirements During Lactation

The principle of 'nutrient partitioning' applies: maternal body mobilizes stores to maintain milk composition — but water-soluble vitamins (B vitamins, vitamin C, iodine) are directly dependent on maternal dietary intake and immediately reflected in milk. Fat-soluble vitamins (D, K) in milk are also responsive to maternal status.

Nutrient	Lactation Requirement & Key Notes
Energy	+340 kcal/day (months 1–6); +400 kcal/day (months 7–12) above non-pregnant, non-lactating. Mobilization of fat stores laid down in pregnancy (fat-burning adaptation) contributes ~170 kcal/day. Weight loss during lactation: 0.5 kg/week acceptable without compromising milk supply. <1,500 kcal/day restricts milk volume
Protein	+25 g/day (total: 71 g/day). Required for synthesis of milk proteins: casein, whey (α -lactalbumin, lactoferrin, immunoglobulins, enzymes). Milk protein content relatively stable regardless of maternal protein intake (protected)
Iodine	290 μ g/day (highest of any life stage). Iodine concentrated in breast milk (active transport); deficiency → infant hypothyroidism + cognitive impairment. WHO recommends universal supplement during lactation. Iodized salt + seafood + dairy are key sources. Seaweed can cause iodine excess — avoid

Vitamin D	600 IU/day for mother; but human milk contains very little vitamin D (12–60 IU/L). AAP and ESPGHAN recommend 400 IU/day direct supplementation for all exclusively breastfed infants. High-dose maternal supplementation (4,000–6,400 IU/day) can sufficiently enrich breast milk — emerging evidence
Vitamin B12	2.8 µg/day. Critical: exclusively breastfed infants of vegan/vegetarian mothers are at high risk of B12 deficiency → irreversible neurological damage. Maternal B12 supplementation immediately enriches milk. All vegan/vegetarian mothers must supplement B12
Choline	550 mg/day (highest of any life stage — 125 mg above pregnancy). Choline concentrated in milk (~160 mg/L). Major source: eggs, meat, fish. Most women do not meet this requirement without targeted food choices or supplementation
DHA	~200–300 mg/day from seafood/algal DHA. DHA in breast milk reflects maternal intake; low-DHA maternal diet → low infant DHA → impaired visual acuity and cognitive development. Oily fish 2–3×/week or algal DHA supplement
Calcium	1,000 mg/day (same as non-pregnant). Bone resorption temporarily increases during lactation (PTHrP from mammary gland → mobilises bone Ca) — maternal BMD decreases 3–9% but fully recovers after weaning. This is a physiological process — additional Ca supplementation does not prevent it

Counseling Tip: *Dietary restrictions during lactation are frequently overstated. Most food components do not significantly alter milk composition or cause infant discomfort. Routine dietary restrictions are not evidence-based. Exceptions: limit caffeine (<200-300 mg/day); avoid high-mercury fish; limit alcohol (safe: feed before drinking, wait ≥2h per standard drink before next feed).*

III. INFANCY & EARLY CHILDHOOD NUTRITION

A. Breast Milk — The Gold Standard for Infant Nutrition

Human breast milk is uniquely and optimally composed for the human infant and represents the reference against which all infant formulas are benchmarked. It provides not only nutrients but a complex biological fluid containing:

- Bioactive components: IgA secretory antibodies (pathogen protection); lactoferrin (iron-binding antimicrobial, anti-inflammatory); lysozyme (antibacterial cell wall lysis); HMOs (Human Milk Oligosaccharides — >200 structures; selectively promote Bifidobacterium infantis; prebiotic; anti-adhesion of pathogens); growth factors (EGF, IGF-1); cytokines; stem cells
- Nutritional composition: Protein ~0.9–1.1 g/100 mL (decreasing as infant ages); Fat ~3.5–4.5 g/100 mL (rich in SFA, MUFA, DHA, AA — AA/DHA ratio important for neurodevelopment); Lactose ~7 g/100 mL (primary carbohydrate — also promotes Ca absorption and bifidogenic bacteria); Vitamins/minerals tuned to infant needs with high bioavailability (Ca absorption ~67% vs ~38% from formula)
- WHO and AAP recommendations: Exclusive breastfeeding for 6 months; continued breastfeeding with complementary foods to 12 months (AAP 2022: 2 years or beyond if mutually desired)

B. Critical Nutrients in Infancy

Nutrient	Requirement, Sources & Critical Notes
Vitamin D	AI: 400 IU/day from birth. Human milk is insufficient (12–60 IU/L). ALL breastfed infants require 400 IU/day supplement from birth. Formula-fed infants (if >1 L/day of vitamin D-fortified formula) may not need additional supplement. Deficiency → rickets, poor bone mineralization, impaired immune function
Iron	AI: 0.27 mg/day (0–6 months — met by breast milk); RDA: 11 mg/day (7–12 months). Foetal iron stores are depleted by ~6 months. Introduction of iron-rich foods (meat, iron-fortified cereals) critical by 6 months. Exclusively breastfed infants should receive 1 mg/kg/day iron supplement from 4 months if complementary foods not introduced. Premature infants: iron supplementation from 1 month (↑ risk of depletion at birth)
Vitamin B12	AI: 0.4 µg/day (0–6m); 0.5 µg/day (7–12m). Foetal stores from mother last ~6 months if mother replete. Vegan mothers with B12 deficiency → infant deficiency → neurodevelopmental damage (often irreversible). Supplement all vegan/vegetarian infants
DHA	AI: ~0.5% of total energy as omega-3 (approximately 70–100 mg/day). Human milk DHA varies with maternal diet (0.06–1.4%). Adequate DHA critical for retinal photoreceptor development and hippocampal synaptogenesis. If formula-fed, choose DHA-supplemented formula
Fluoride	No supplement <6 months. AI: 0.01 mg/day (0–6m); 0.5 mg/day (6–12m). If household water fluoride <0.3 ppm: supplement 0.25 mg/day from 6 months (primary prevention of dental caries)
Zinc	AI: 2 mg/day (0–6m); RDA: 3 mg/day (7–12m). Growth, immune function, cognitive development. Colostrum is high in zinc (15 mg/L → decreases to 1 mg/L in mature milk). First foods should include zinc-rich sources (meat, legumes, zinc-fortified cereals)

C. Complementary Feeding — Timing, Sequence & Approach

- Initiation: 6 months (WHO recommendation); 4–6 months window acknowledged by ESPGHAN if developmental readiness present (sits with support, shows interest, lost tongue-thrust reflex). Earlier introduction does NOT improve sleep or growth and increases allergy/obesity risk
- First foods: Iron-rich foods as priority (pureed/minced meat, poultry, fish, iron-fortified cereals, legumes); vegetables and fruits; no added salt or sugar; no honey (Clostridium botulinum spore risk in <12 months); no cow's milk as main drink <12 months (insufficient iron, inappropriate renal solute load)
- Allergen introduction: Current evidence (LEAP trial, EAT study): early introduction (4–6 months) of allergenic foods (peanuts, eggs, tree nuts, fish, wheat) while breastfeeding continues REDUCES risk of food allergy development — opposite of prior 'avoidance' recommendations. Introduce one new food at a time with 3–5 day observation for reaction
- Baby-led weaning (BLW): Soft, age-appropriate finger foods from 6 months alongside traditional purees; promotes self-regulation, texture acceptance, family mealtime integration. Safety: ensure soft textures; choking hazards (whole grapes, raw carrots, nuts) avoided. Nutritional concern: iron intake must be monitored — iron-rich finger foods prioritized

IV. ADULTHOOD NUTRITION

A. General Nutrient Requirements — Young to Middle Adult

Nutritional requirements during adulthood (19–50 years) are generally the most stable of the life cycle, representing the physiological 'baseline' against which other life stages are compared. Key priorities shift from growth-support to chronic disease prevention and maintenance of health:

- Energy: Total energy expenditure (TEE) calculated using REE × physical activity level (PAL). TEE declines ~50–100 kcal/decade after age 30 due to ↓ lean mass and ↓ activity. US adults: average energy intake ~2,100 kcal/day (women), ~2,600 kcal/day (men)
- Macronutrient distribution (AMDR): Carbohydrate 45–65%; Fat 20–35%; Protein 10–35% of total energy. Protein RDA: 0.8 g/kg/day — likely insufficient for active adults; emerging evidence supports 1.2–1.6 g/kg/day for lean mass preservation in middle adulthood
- Fibre: AI 25 g/day (women), 38 g/day (men). Average US intake: ~15 g/day — significant shortfall. Increase from whole grains, legumes, vegetables, fruits
- Micronutrient priorities — sex-specific: Women (19–50): Iron 18 mg/day (menstrual losses); Folate 400 µg DFE (periconceptual risk); Calcium 1,000 mg/day. Men (19–50): Selenium 55 µg/day; Magnesium 400–420 mg/day; Zinc 11 mg/day

B. Chronic Disease Prevention Through Dietary Pattern

Dietary Pattern	Key Features & Chronic Disease Evidence
Mediterranean Diet	High EVOO, vegetables, fruits, legumes, whole grains, fish, nuts; moderate wine; low red/processed meat. Strongest evidence base: PREDIMED trial: 30% ↓ CVD events; inversely associated with T2DM, cognitive decline, all-cause mortality. UNESCO Intangible Cultural Heritage
DASH Diet	↑ Fruits, vegetables, low-fat dairy, whole grains, lean proteins, nuts, legumes; ↓ Na ⁺ , saturated fat, added sugars. Most clinically validated antihypertensive dietary intervention: ↓ SBP 8–14 mmHg in hypertensive individuals; strong evidence for T2DM prevention
Plant-Based / Whole Food Plant-Based	Emphasizes whole plant foods; varies from flexitarian to vegan. Inversely associated with obesity, T2DM, CVD, colorectal cancer. Key: ensure adequate B12, iron, zinc, omega-3 DHA — covered in vegetarian section
Dietary Inflammatory Index (DII)	Quantifies overall dietary inflammatory potential. Pro-inflammatory diet (↑ DII): associated with ↑ CRP, ↑ CVD, ↑ cancer, ↑ T2DM. Anti-inflammatory diet features: omega-3, polyphenols, fibre, turmeric, ginger, garlic; ↓ refined carbohydrates, processed meat, trans fat

V. ELDERLY NUTRITION

A. Physiological Changes Affecting Nutritional Status in Ageing

Physiological Change	Nutritional Consequence & Intervention
↓ Appetite (anorexia of ageing)	↓ energy intake → involuntary weight loss. Mechanisms: ↑ CCK satiety signaling; ↑ leptin sensitivity; ↓ ghrelin; delayed gastric emptying; sensory decline (smell/taste). Intervention: small frequent meals, energy-dense foods, oral nutritional supplements (ONS) if intake <75% estimated needs
↓ Gastric acid (achlorhydria ~30–40% elderly)	Impairs absorption of: vitamin B12 (requires acid for peptic release from food protein; crystalline B12 supplements bypass this — absorbed normally), iron ($\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$ requires acid), Ca^{2+} (CaCO_3 requires acid → CaCitate preferred supplement in achlorhydria), zinc. PPI users at additional risk
↓ Renal 1α -hydroxylase activity	↓ calcitriol synthesis → ↓ Ca^{2+} absorption → ↑ PTH → secondary hyperparathyroidism → bone loss. Vitamin D requirement increases: 800 IU/day (>70 years DRI); many elderly need 1,000–2,000 IU to achieve $25(\text{OH})\text{D} \geq 75 \text{ nmol/L}$
↓ Skin 7-dehydrocholesterol	↓ vitamin D_3 synthesis from UVB — elderly produce only ~25% as much vitamin D from equivalent sun exposure as young adults. Reinforces importance of dietary/supplemental vitamin D
↓ Thirst sensation + impaired ADH response	↑ dehydration risk — elderly do not perceive thirst reliably. ↑ risk of hypernatraemia, UTI, confusion, medication toxicity. Counsel: schedule fluid intake rather than relying on thirst
↓ Muscle protein synthesis response (anabolic resistance)	Same protein dose produces less MPS than in young adults. Requires higher protein per meal to stimulate MPS maximally: 30–40g/meal (vs 20–25g in young adults). Leucine supplementation (2.5–3g leucine per meal) may help overcome anabolic resistance. Protein RDA (0.8 g/kg/day) likely insufficient — PROT-AGE Study Group recommends 1.0–1.2 g/kg/day for healthy elderly; 1.2–1.5 g/kg/day if disease or injury present
↓ Intestinal transit → constipation	Fluid intake $\geq 1.5 \text{ L/day}$ + dietary fibre 20–25 g/day + physical activity. Adequate fluid essential — inadequate fluid with high fibre can worsen constipation
Polypharmacy drug-nutrient interactions	Warfarin-vitamin K; metformin-B12 (↓ absorption); proton pump inhibitors-B12, Ca, Mg; thiazide diuretics-K, Mg, Zn; loop diuretics-K, Ca, Mg; long-term corticosteroids-Ca, vitamin D, protein (catabolic); cholestyramine-fat-soluble vitamins; methotrexate-folate

B. Key Nutrient Recommendations for Elderly (>65 Years)

Nutrient	DRI (>70 years)	Priority Rationale
Protein	1.0–1.2 g/kg/day (above RDA)	Sarcopenia prevention; anabolic resistance; 30–40g/meal with leucine-rich sources (whey, egg, lean meat)
Vitamin D	800 IU/day (DRI); many experts 1,000–2,000 IU	↓ falls (muscle function), bone, immune; ↓ skin synthesis; achlorhydria; institutionalised elderly often severely deficient
Calcium	1,200 mg/day	Bone mineral maintenance; post-menopausal bone loss; Ca-citrate preferred if achlorhydria; food sources preferred over supplements
Vitamin B12	2.4 µg/day (as crystalline — not food-bound)	Achlorhydria → ↓ food-bound B12 absorption. Sublingual, IM, or high-dose oral crystalline B12 supplement indicated
Folate	400 µg DFE/day	One-carbon metabolism; cognitive decline association; dementia risk mitigation; ensure B12 adequate first (masking)
Potassium	2,600–3,400 mg/day	BP control; DASH diet foundation; often deficient in restricted/institutionalized elderly
Omega-3 (EPA+DHA)	250–500 mg/day	Anti-inflammatory; CVD protection; cognitive decline attenuation (mixed evidence); muscle protein synthesis in sarcopenia
Magnesium	320–420 mg/day	Often deficient (↓ intake + ↑ renal loss); >300 enzyme reactions; insulin sensitivity; sleep; muscle cramps

Clinical Note: *Malnutrition Screening in Elderly: Mini Nutritional Assessment (MNA) — 18-point validated tool; MUST (Malnutrition Universal Screening Tool) — rapid 5-minute assessment. Malnutrition (MNA score <17) affects ~30% of hospitalized elderly, ~60% of nursing home residents. Consequences: ↑ pressure ulcer risk, ↑ infections, ↑ falls, prolonged hospitalisation, ↑ mortality. Oral nutritional supplements (ONS): 400–600 kcal/day of high-protein, micronutrient-complete supplement reduces mortality and hospital readmission in malnourished elderly (Cochrane meta-analysis).*

VI. VEGETARIAN & VEGAN NUTRITION

A. Spectrum of Plant-Based Dietary Patterns

Pattern	Foods Excluded	Key Nutritional Considerations
Flexitarian / Semi-vegetarian	Occasional meat	Minimal restriction; generally nutritionally complete
Pesco-vegetarian / Pescatarian	All meat except fish	EPA/DHA well supplied; B12 from fish; virtually no nutritional concerns
Lacto-ovo-vegetarian	All meat and fish; includes dairy and eggs	B12 from dairy/eggs; iron and zinc need attention; DHA moderate concern
Lacto-vegetarian	All meat, fish, and eggs; includes dairy	Similar to above; choline from dairy limited; B12 from dairy
Ovo-vegetarian	All meat, fish, dairy; includes eggs	B12 from eggs (limited bioavailability); Ca must come from plant sources
Vegan	All animal products	Multiple nutrients require supplementation/strategic planning (B12, D3, DHA, iodine, Ca, Fe, Zn, choline — see below)
Raw vegan	All animal products + all cooked foods	Severe restriction; high deficiency risk; B12 deficiency essentially certain without supplementation; not recommended for pregnancy, infancy, children, elderly

B. Critical Nutrients in Vegetarian/Vegan Diets — Risk & Solutions

Nutrient	Risk Level & Reason	Dietary Strategy & Supplementation
Vitamin B12	CRITICAL — Deficiency certain in strict vegans without supplementation. No reliable plant source (nori, fermented foods, spirulina have analogues that do NOT function as B12 and may block absorption). Deficiency: irreversible neurological damage (subacute combined degeneration), megaloblastic anaemia	Supplement: 2,000–2,500 µg cyanocobalamin weekly OR 25–100 µg/day daily (higher dose overcomes reduced absorption efficiency). Methylcobalamin also effective. B12-fortified foods (plant milks, nutritional yeast) can contribute but supplementation is the only reliable strategy. Verify with serum B12 + MMA annually
Vitamin D	High — Dietary D ₃ only in animal foods; D ₂ (ergocalciferol, plant) available but less potent. Mushrooms UV-exposed = variable D ₂ . Sun exposure unreliable in many populations	Supplement with D ₃ (lichen-derived vegan D ₃ available) or D ₂ : 1,000–2,000 IU/day. Monitor 25(OH)D; target ≥75 nmol/L. Critical in vegan pregnancy, infancy, elderly

Omega-3 DHA/EPA	High — No preformed EPA/DHA in plants. ALA (walnuts, flaxseed, chia) poorly converted to EPA (~5–10%) and DHA (<1%). Vegan DHA levels significantly lower than omnivores	Algal DHA/EPA supplement (sourced from the same microalgae that fish eat — biologically identical, mercury-free): 200–500 mg DHA/day. Essential for vegan pregnancy, lactating mothers, and infants. Daily consumption of ALA-rich foods also recommended
Iron	Moderate-High — Only non-haeme iron in plant foods (2–20% bioavailability vs 15–35% haem iron). Phytate, tannins, calcium all inhibit. Vegans have lower ferritin but comparable haemoglobin — may reflect adaptation	Iron RDA adjusted upward 1.8× for vegetarians/vegans (32 mg/day women, 14 mg/day men). Strategies: vitamin C with every iron-rich meal (↑ absorption 2–4×); consume legumes, tofu, fortified cereals, seeds; avoid tea/coffee with meals; soak and sprout legumes (↓ phytate); cook in cast-iron cookware (minor contribution). Monitor ferritin + TIBC at least annually
Zinc	Moderate — Zinc in plants bound by phytate (↓ bioavailability ~50% vs omnivore diet). Highest plant sources: pumpkin seeds, hemp seeds, chickpeas, cashews, oats	Zinc RDA adjusted 1.5× for vegetarians. Strategies: soak legumes and seeds; fermentation/yeast leavening (↓ phytate); eat sprouted grains; consider zinc supplement (8–11 mg/day) if monitoring shows deficiency. Zinc status: serum Zn + symptom assessment
Calcium	Moderate — Dairy-free vegans must source entirely from plants. High-bioavailability plant sources: bok choy, kale, broccoli, fortified plant milks. Low-bioavailability: spinach, beet greens (high oxalate)	Target 1,000–1,200 mg/day. Priority sources: fortified plant milks (320 mg/250 mL — choose fortified); calcium-set tofu (350 mg/100g); bok choy (105 mg/100g, ~50% absorption); fortified orange juice; white beans. Supplement Ca-citrate (300–500 mg twice daily with meals) if dietary intake insufficient. Shake fortified plant milks well (calcium settles)
Iodine	HIGH — Dairy and seafood are major dietary iodine sources. Plant foods have highly variable iodine content depending on soil iodine. Seaweed: wildly variable (50–8,000 µg/g) — may cause excess rather than deficiency	Use iodised salt consistently (1/4 tsp = ~95 µg iodine). Supplement: 150 µg/day potassium iodide. Critical in vegan pregnancy/lactation (290 µg/day needed). Avoid relying on seaweed due to extreme variability and excess risk. UIC monitoring in vegan pregnant women recommended
Choline	Moderate — Eggs and meat are dominant dietary sources. Plant sources limited: soybeans (107 mg/100g), potatoes (12 mg/100g), nuts, cruciferous vegetables (low in choline)	Vegan choline intake often 50–60% below AI. Strategy: 3 cups soybeans/day theoretically meets needs but impractical. Choline supplement (200–300 mg/day as choline bitartrate) recommended for vegan women, especially in pregnancy/lactation. Lecithin (phosphatidylcholine) from sunflower also available

C. Health Outcomes of Vegetarian/Vegan Diets — Evidence Summary

Health Outcome	Evidence & Effect Size
Cardiovascular disease	Well-planned vegetarian/vegan diets associated with 20–30% lower CVD risk (Adventist Health Study-2, EPIC-Oxford). Lower BMI, LDL-C, blood pressure, inflammatory markers. Greatest benefit for CHD; less clear for stroke (may be higher in some vegans — possibly B12-related homocysteine)
Type 2 diabetes	Vegetarian diets associated with 23–26% lower T2DM risk (AHS-2). Vegan diets show strongest association (RR ~0.67). Mechanisms: lower BMI, higher fibre, lower saturated fat, polyphenol content
Cancer	WCRF: Higher fibre intake (predominantly from plant foods) → ↓ colorectal cancer. Meta-analyses: vegetarians have 8–18% lower overall cancer incidence. Evidence strongest for GI cancers
Bone health	Vegans have modestly lower BMD and higher fracture risk than omnivores (EPIC-Oxford Bone fracture study: vegans 43% higher fracture risk). Primarily explained by lower calcium and protein intakes — largely preventable with adequate intake strategies
Longevity	Adventist vegetarians among the longest-lived populations globally (Blue Zone — Loma Linda, CA). Median life expectancy ~7–10 years longer than general US population. Diet, community, stress management, and non-smoking compound
Pregnancy outcomes	Well-planned vegan pregnancy with appropriate supplementation (B12, D, DHA, iodine, Fe) can achieve normal foetal growth and development. Risk: inadequately supplemented vegan mothers → high rates of infant B12 deficiency, low DHA, iodine deficiency. Registered Dietitian supervision strongly recommended for vegan pregnancy

Counseling Tip: *The Academy of Nutrition and Dietetics (2016 Position Paper) states: 'Well-planned vegetarian, including vegan, diets are healthful, nutritionally adequate, and may provide health benefits for the prevention and treatment of certain diseases.' Key qualifier: 'well-planned.' RD involvement is essential, particularly for pregnancy, infancy, children, and elderly vegan populations.*

Lecture 2 Summary — Key Principles

- **Pregnancy:** most demanding for folate (NTDs — supplement 400–600 µg preconceptionally), iron (27 mg/day — screen ferritin at booking and 28 weeks), iodine (220 µg/day — brain development), DHA (~200–300 mg/day — foetal brain accretion), and choline (450 mg/day — underrecognised)
- **Lactation:** most demanding life stage for iodine (290 µg/day), choline (550 mg/day), and energy (+340–400 kcal/day); water-soluble vitamins and DHA in breast milk directly reflect maternal diet. ALL breastfed infants need 400 IU/day vitamin D supplement from birth
- **Infancy:** early allergen introduction (4–6 months) reduces allergy risk; iron and vitamin D supplementation for breastfed infants; complementary foods prioritize iron-rich options at 6 months
- **Elderly:** anorexia of ageing, achlorhydria, ↓ thirst, polypharmacy, anabolic resistance, and ↓ skin vitamin D synthesis create multiple concurrent deficiency risks. Protein 1.0–1.2 g/kg/day; vitamin D 800–2,000 IU/day; crystalline B12 supplement; Ca-citrate preferred

- Vegetarian/Vegan: B12 supplementation is MANDATORY for strict vegans (not negotiable — no reliable plant source); algal DHA/EPA for omega-3; iodized salt/supplement for iodine; iron strategies (vitamin C + absorption enhancement); calcium from low-oxalate sources; choline supplement in pregnancy/lactation
- Overall principle: Physiological changes at each life stage alter both nutrient requirements and absorption/metabolism — static RDAs must be interpreted in the context of the individual's physiological state

Suggested Reading:

1. Picciano MF (2003). Pregnancy and lactation: physiological adjustments, nutritional requirements and the role of dietary supplements. J Nutr. |
2. Koletzko B et al. (2019). Nutrition during pregnancy, lactation and early childhood. Ann Nutr Metab. |
3. Rogerson D (2017). Vegan diets: practical advice for athletes and exercisers. J Int Soc Sports Nutr. |
4. Dent E et al. (2018). Malnutrition in hospitalised older adults. World J Gastroenterol.

LECTURE 3

Food Safety & Adequacy

Food Chain Impact, Processing, Storage, Food Toxicants & Allergies on Nutritional Quality

Nutrition & Dietetics MS Program | Nutritional Assessment & Specialized Needs Series

Learning Outcomes — By the end of this lecture, students will be able to:

- Trace the farm-to-fork food chain and identify points of nutritional and safety vulnerability at each stage
- Explain the biochemical mechanisms by which processing and cooking alter macronutrient and micronutrient content
- Evaluate the nutritional consequences of industrial food processing, including fortification and enrichment strategies
- Describe the major categories of food toxicants (natural, processing-derived, environmental) and their mechanisms of harm
- Classify food allergies by immunological mechanism and describe the clinical management of major food allergies
- Apply food safety and adequacy principles to dietary counselling for individuals with food allergies, intolerances, and toxicant exposures

I. THE FOOD CHAIN — FARM TO FORK

A. Stages of the Food Chain & Nutritional Vulnerabilities

The food chain encompasses all stages from primary production through processing, distribution, retail, home preparation, and consumption. Each stage introduces potential for nutritional gain or loss, contamination, and safety hazards:

Stage	Key Nutritional/Safety Concerns	Critical Interventions
Primary production (Farming/Growing)	Soil nutrient depletion → ↓ mineral content of produce over decades (selenium, iodine, zinc — highly soil-dependent). Pesticide/herbicide residues. Veterinary drug residues (antibiotics, growth hormones). Heavy metal uptake from soil (cadmium in wheat, lead, arsenic in rice). GMO crops — nutritional modification (Golden Rice: β-carotene biofortification). Organic vs conventional: minimal micronutrient difference; lower pesticide residue in organic	Biofortification (agronomic: Se-enriched fertilizers; plant breeding: iron/zinc-biofortified crops — HarvestPlus program). Soil testing and mineral supplementation of soil. Good Agricultural Practice (GAP) standards. Pesticide maximum residue limits (MRLs) — Codex Alimentarius, EU, FDA
Post-harvest handling & storage	Vitamin C loss: 15–50% in 1–7 days at room temperature; ↓ with refrigeration. Carotenoid degradation by light/oxygen. Lipid oxidation in nuts/seeds (rancidity). Enzyme-mediated breakdown: polyphenol oxidase (enzymatic	Cold chain management (refrigeration ≤4°C; frozen ≤-18°C). Modified atmosphere packaging (↑ CO ₂ , ↓ O ₂). Antioxidant coatings (ascorbic

	browning in fruit), lipoxygenase (off-flavours in legumes). Mycotoxin contamination (Aspergillus → aflatoxin; Fusarium → deoxynivalenol/DON, fumonisins) in improper grain/nut storage	acid, citric acid). Moisture control (<13% for grains to prevent mycotoxin). HACCP (Hazard Analysis Critical Control Point) implementation
Processing & Manufacturing	Nutrient losses (heat, water, oxygen — see section II). Addition of additives (preservatives, colourings, emulsifiers, flavour enhancers). Acrylamide formation (high-temperature starchy foods). Trans fat from partial hydrogenation (largely eliminated post-FDA ban). AGE formation in heated/ultra-processed foods. NOVA classification: Group 4 ultra-processed foods → ↑ CVD, T2DM, cancer risk in large cohort studies	Mandatory fortification (iodization, iron, folate, vitamin D in staple foods). Process optimization to minimize nutrient loss. Reduction of additives of concern (sodium, saturated fat, added sugar — nutrient profile scoring: Nutri-Score, HFSS classification). Trans fat elimination (FDA GRAS revocation)
Distribution & Retail	Cold chain breaks → microbial growth (Listeria in chilled RTE, Salmonella in raw poultry, E. coli O157 in fresh produce). Date labeling confusion ('best before' vs 'use by'). Food deserts: geographic inequity in access to fresh produce/nutritious food — a primary driver of diet-related health disparities	Food date labeling standardization. Food bank donations from retailers (Good Samaritan laws). SNAP (food assistance) at farmers' markets. Food environment interventions — shelf-placement, product reformulation, front-of-pack labeling (traffic light systems)
Home preparation & consumption	Cooking nutrient losses (see section II). Cross-contamination (raw meat → produce). Undercooking of meat/poultry/eggs (pathogen survival). Storage of leftovers >2h at room temperature → bacterial exponential growth (danger zone 5–60°C). Portion distortion — increased plate/portion sizes over decades → passive overconsumption	Food safety education: the '4 Cs' (Clean, Separate, Cook, Chill). Safe internal temperatures (poultry 74°C, ground meat 71°C, seafood 63°C). Food thermometer use. USDA MyPlate guidance on portion sizes

II. FOOD PROCESSING — NUTRITIONAL CONSEQUENCES

A. Effect of Thermal Processing on Nutrients

Nutrient Class	Thermal Sensitivity & Specific Effects
Vitamin C (ascorbic acid)	Most labile vitamin — oxidized by O ₂ , degraded by heat, leached in water. Losses: boiling 40–70%; steaming 10–25%; microwaving <15%. Oxidation accelerated by Cu ²⁺ and Fe ³⁺ in cooking vessels (avoid copper pans). Key strategies: minimal water, short cooking time, steam/microwave, consume raw where possible
Folate	Heat-labile; leaches into cooking water; oxidized. Losses: boiling vegetables 40–70%; blanching before freezing 15–30%. Fortification essential in populations where cooking destroys much dietary folate (US mandatory fortification of enriched grain products since 1998 → 26% ↓ NTD prevalence)
Thiamin (B1)	Highly heat-sensitive; alkaline conditions accelerate destruction; leaches into cooking liquid. Losses: boiling meat 50–80%; bread baking 25–30%. Thiamin enrichment of refined flour is mandatory in many countries (replaces what is removed by milling)
Vitamins B2, B6, Pantothenic Acid	Moderate heat sensitivity; variable leaching. Riboflavin highly sensitive to UV light (milk in clear glass bottles loses 70% B2 in 2 hours of direct sunlight — use opaque/cardboard containers). B6 losses: boiling vegetables 30–50%; canning 50–70%
Fat-Soluble Vitamins (A, D, E, K)	Relatively heat-stable but vulnerable to oxidation and light. Vitamin E most sensitive to oxygen — decreases significantly during deep frying. Vitamin A stable in most cooking but destroyed by high-temperature drying. Bioavailability of carotenoids (provitamin A) often INCREASES with heating (cell wall disruption → ↑ release)
Proteins	Denaturation begins at 40°C — changes tertiary structure but does not reduce nutritional value unless prolonged high heat. Benefit: denaturation improves digestibility (unfolded proteins more accessible to proteases). Maillard reaction (reducing sugars + amino acid side chains, especially lysine) at >140°C → glycated proteins with ↓ lysine bioavailability; produces flavour compounds and AGEs; excessive Maillard → nutritional loss
Lipids	Oxidation (rancidity) accelerated by high heat, repeated use, oxygen, light, metals. Deep frying: repeated use → ↑ polar compounds, ↑ trans FA formation, ↑ polymerization products, ↑ cyclic fatty acid monomers (CFAMs). Healthy fats (EVOO) degraded by deep-frying temperatures (>180°C). Omega-3 (EPA/DHA) most vulnerable to oxidation
Dietary Fibre	Generally heat-stable; water-soluble fibre may leach into cooking liquid. Soluble fibre (pectin) undergoes hydrolysis at very high temperatures → ↓ viscosity and ↓ LDL-lowering efficacy. Resistant starch: cooking and cooling increases RS3 content (retrograde starch) — cooked and cooled rice/potatoes/pasta have significantly higher RS3 than freshly cooked (beneficial for glycaemic index and colonic fermentation)

B. Industrial Processing — The NOVA Classification System

The NOVA framework (Monteiro et al., 2010, 2019 — University of São Paulo) classifies foods by extent and purpose of processing — a public health tool with strong epidemiological evidence:

NOVA Group	Definition	Examples	Health Implications
Group 1 Unprocessed/ Minimally Processed	Natural foods altered minimally (drying, cooling, fermenting) without addition of other substances	Fresh fruit, vegetables, meat, fish, milk, plain yogurt, dried beans, eggs, whole grains (plain)	Nutritional reference; preserves bioactive compounds; epidemiologically associated with lowest chronic disease risk
Group 2 Processed Culinary Ingredients	Derived from Group 1 by pressing, grinding, refining, milling — used to prepare Group 1 foods	Oils, butter, flour, pasta, salt, sugar, vinegar, dried herbs	Not consumed alone; nutrient density varies; refined products lose fibre and micronutrients
Group 3 Processed Foods	Group 1 + Group 2 + often salt, sugar, or oil added to preserve or enhance — recognizable as modified versions of Group 1	Canned vegetables (with salt), cured meats, cheeses, freshly baked bread, salted nuts, canned fish in oil	Generally adequate nutritional quality; some concerns with sodium, saturated fat in processed meats
Group 4 Ultra-Processed Foods (UPF)	Industrial formulations with ≥5 ingredients incl. additives (emulsifiers, flavour enhancers, colour, sweeteners, preservatives); little or no whole food content	Soft drinks, packaged snacks, reconstituted meat products, instant noodles, breakfast cereals with additives, fast food, flavoured milks	Strong epidemiological associations: ↑ CVD, T2DM, obesity, colorectal cancer, depression, all-cause mortality (multiple large cohort studies, NutriNet-Santé, UK Biobank, PREDIMED-Plus)

Mechanisms of UPF harm are debated — likely multifactorial: hyper-palatability (engineered sweetness, saltiness, fat combinations exceed natural food stimuli → overconsumption); ultra-rapid digestion of refined starches/sugars → high glycaemic response; displacement of fibre and phytonutrients; additives (emulsifiers may disrupt intestinal mucosa — carrageenan, CMC, polysorbate 80 in animal studies → ↑ intestinal permeability, dysbiosis); food matrix destruction; high energy density.

C. Food Fortification & Enrichment

Type	Definition & Examples
Mandatory fortification	Government-mandated addition of nutrients to specific foods to address population-wide deficiency. US examples: iodization of salt (1924, goitre elimination); iron + thiamin + riboflavin + niacin + folate enrichment of refined grain products (1943 + folate 1998); vitamin D fortification of milk (1930s, rickets elimination); vitamin A + D addition to margarine. Global: WHO/FAO recommend iron + folic acid fortification of wheat flour (190+ countries). MOST SUCCESSFUL public health nutrition intervention globally
Voluntary/optional fortification	Manufacturer-added nutrients for marketing/consumer appeal. Examples: breakfast cereals (often 25–100% DV multiple vitamins); fortified plant milks (Ca ²⁺ , B12, D, iodine — critical for vegans); fortified juices (vitamin C, calcium); energy drinks (B vitamins — often

	pharmacological doses). Concern: selective fortification of nutritionally poor foods ('nutrient profiling' challenge — 'junk food' fortification)
Bio fortification	Breeding or genetic modification of crops to have higher micronutrient content. HarvestPlus program examples: provitamin A cassava and sweet potato (sub-Saharan Africa); iron/zinc rice and wheat (South/Southeast Asia); zinc maize; folate tomatoes. Reaches rural populations who do not access processed/fortified foods. Cost-effective, sustainable
Restoration/Enrichment	Adding back nutrients lost during processing to the level present in the unprocessed food. Example: vitamin C added to canned tomatoes; vitamins added back to UHT milk (processing heat destroys); B vitamins added back to milled white rice (brown rice reference)

III. FOOD TOXICANTS

A. Naturally Occurring Food Toxicants

Toxicant / Category	Source, Mechanism & Clinical Significance
Glycoalkaloids (Solanine, Chaconine)	Nightshade family: potatoes (highest in sprouts, green skin, damaged areas), green tomatoes, aubergine. Mechanism: inhibit acetylcholinesterase → cholinergic excess; disrupt cell membranes → haemolysis. Safe threshold: <20 mg/100g potato (green potatoes: up to 80 mg/100g — toxicity risk). Management: discard green and sprouted potatoes; do not eat potato peel from green potatoes. Cooking only partially reduces content
Cyanogenic Glycosides (Linamarin, Amygdalin)	Cassava (linamarin — major staple for 600 million people globally), bitter almonds, apple/cherry/apricot seeds (amygdalin — not in flesh). Metabolism by β -glucosidase (in gut or in food by enzyme) → HCN (hydrogen cyanide). Cassava: inadequate processing → chronic low-level HCN exposure → tropical ataxic neuropathy (konzo) in Africa. Management: proper cassava processing (peeling, soaking, fermenting, sun-drying — reduces HCN >90%). Apricot/cherry stones: avoid consuming
Lectins	Kidney beans (phytohaemagglutinin — PHA), soybeans, peanuts, wheat (WGA). Lectins bind glycoproteins on GI mucosa → agglutinate RBCs, disrupt intestinal epithelium, impair nutrient absorption. Raw kidney bean poisoning: 4–5 beans sufficient → nausea, vomiting, diarrhoea within 3 hours. Management: mandatory soaking (8h+) and full boiling (≥ 10 min at rolling boil — slow cookers insufficiently hot → may INCREASE toxicity). Canning destroys lectins. Moderate concern for raw plant-based diets (fermentation ↓ content)
Oxalates	Spinach, Swiss chard, beet greens, rhubarb, cocoa. Bind Ca^{2+} , Mg^{2+} , Fe^{2+} in gut → ↓ mineral absorption (spinach Ca absorption ~5%). Soluble oxalate (sodium oxalate) absorbed → forms calcium oxalate crystals → renal stones (calcium oxalate urolithiasis — most common kidney stone type). Clinical: low-oxalate diet for recurrent kidney stone formers (<50 mg/day); adequate calcium intake (binds oxalate in gut → ↓ absorption)

Phytic Acid (Phytate)	Whole grains, legumes, nuts, seeds. Chelates Fe^{2+} , Zn^{2+} , Ca^{2+} , Mg^{2+} , $\text{Mn}^{2+} \rightarrow \downarrow$ bioavailability (up to 50% reduction). Concern for populations consuming primarily unprocessed plant foods. Management: soaking (activates phytase enzymes), sprouting, fermentation (lactic acid bacteria produce phytase $\rightarrow$ hydrolyses phytate), yeast leavening (bread). Not eliminated by cooking alone
Goitrogens	Glucosinolate breakdown products (thiocyanates) in cruciferous vegetables; flavonoids in soy (genistein inhibits TPO). Mechanism: thiocyanates compete with iodine at NIS transporter; isoflavones inhibit thyroid peroxidase (TPO) $\rightarrow \downarrow$ thyroid hormone synthesis. Clinically significant only in iodine-deficient individuals or those with very high cruciferous vegetable consumption. Cooking reduces goitrogen content (myrosinase denaturation $\rightarrow$ fewer breakdown products). Not a concern in iodine-replete populations
Biogenic Amines (Histamine, Tyramine)	Histamine: spoiled/fermented fish, aged cheese, red wine, fermented sausages (from bacterial histidine decarboxylase). Tyramine: aged cheese, red wine, soy sauce, fermented meats. Mechanism: histamine $\rightarrow$ pseudoallergic reaction (mimics IgE-mediated allergy without sensitization); symptoms: flushing, urticaria, headache, GI distress. Tyramine: interacts with MAO inhibitor antidepressants $\rightarrow$ tyramine syndrome (hypertensive crisis). Management: strict low-tyramine diet for patients on MAOIs; histamine intolerance (DAO enzyme deficiency) — low-histamine diet

B. Processing-Derived Toxicants

Toxicant	Formation & Health Concern
Acrylamide	Formed from asparagine + reducing sugars via Maillard reaction at temperatures $>120^{\circ}\text{C}$ in starchy foods (crisps, chips, biscuits, bread, coffee roasting, breakfast cereals). IARC Group 2A carcinogen (probably carcinogenic to humans — based on animal studies; epidemiological evidence inconclusive). EU food safety authorities set indicative values and reduction strategies (ALARA principle). Reduction: prefer boiling/steaming over frying/roasting; avoid overcooking starchy foods; light golden colour target for toast/chips ('Go for gold' campaign)
Heterocyclic Amines (HCAs) Polycyclic Aromatic Hydrocarbons (PAHs)	HCAs: formed from creatine + amino acids + sugars in muscle meat at high temperature ($>150^{\circ}\text{C}$) — pan-frying, grilling, barbecuing. Examples: PhIP, IQ, MeIQx. Mutagenic (Ames test positive); IARC Group 2A. PAHs: formed by incomplete combustion during charcoal/wood grilling; fat drips onto heat source $\rightarrow$ smoke deposits PAHs on food surface. IARC Group 1 carcinogen (benzo[a]pyrene). Reduction: lower temperature cooking; avoid charring; microwave pre-cooking before grilling; flip meat frequently; trim fat; avoid direct flame contact with food; marinating ($\downarrow$ HCA by $\sim 70\%$ — antioxidants in marinade)
Trans Fatty Acids (industrial)	Formed by partial hydrogenation of vegetable oils — solid fats for margarine, shortening, processed foods. Industrial trans FA (elaidic acid — 18:1 trans) $\uparrow$ LDL-C, $\downarrow$ HDL-C, $\uparrow$ inflammation $\rightarrow$ strongest dietary risk factor for CVD (2% $\uparrow$ energy from trans FA $\rightarrow$ 23% $\uparrow$ CHD risk). FDA revoked GRAS status 2015 $\rightarrow$ PHOs banned in US food supply by 2018. WHO 'REPLACE' program targets global elimination

	by 2023. Ruminant trans FA (vaccenic acid, CLA from dairy/meat) appear neutral or beneficial — distinct from industrial trans FA
Advanced Glycation End Products (AGEs)	Non-enzymatic glycation during high-heat cooking (Maillard reaction) → CML, pentosidine, methylglyoxal adducts. Highest in dry heat-cooked animal products (roasted/grilled meat, cheese, butter) vs boiled/poached equivalents (3-10× lower AGE content). Absorbed AGEs activate RAGE (Receptor for AGEs) → NF-κB → systemic inflammation; associated with accelerated ageing, CVD, diabetic complications, CKD progression. Reduction: lower cooking temperatures, more water-based cooking, acidic marinades (↓ AGE by ~50%)
Nitrosamines (N-nitroso compounds)	Formed from nitrites (used as preservatives in cured/processed meats: bacon, hot dogs, deli meats) + secondary amines from amino acids during cooking. N-nitrosamine formation accelerated at high temperatures. Vitamin C and E inhibit nitrosamine formation (added to cured meats for this reason). IARC: processed meats Group 1 carcinogen; WCRF: each 50g/day processed meat → 16% ↑ colorectal cancer risk. Counsel: limit processed meat; choose lower-nitrite products; cook at lower temperatures; consume vitamin C alongside
Furan	Formed during heat processing of carbohydrates in closed systems (canning, UHT processing, coffee roasting). Volatile — predominantly escapes on opening. Animal carcinogen (IARC Group 2B). Highest in jarred baby food with meat — EFSA recommends reducing infant exposure. Open cans/jars before heating and stir to allow furan to escape

C. Environmental Contaminants in Food

Contaminant	Sources, Accumulation & Health Impact
Mercury (methylmercury)	Industrial pollution → aquatic methylation by bacteria → bioaccumulates in marine food chain (highest in large predatory fish: shark, swordfish, king mackerel, tilefish, albacore tuna). Neurotoxin — binds selenium (depletes antioxidant selenoproteins), crosses BBB and placenta. Most harmful to developing brain: impairs hearing, vision, language, coordination. FDA/EPA guidance: pregnant women limit albacore tuna (≤170g/week), avoid 4 high-mercury species; lower mercury choices: salmon, shrimp, canned light tuna, sardines, tilapia
Lead (Pb)	Old paint (pre-1978 US housing) → dust/soil ingestion by children. Lead pipes in water distribution. Some ceramic glazes, traditional medicine preparations. Children most vulnerable: absorb 40–50% vs adults 10%. No safe blood lead level — neurotoxic at any level (impairs cognitive development, IQ). Nutritional protection: adequate Ca (↓ intestinal Pb absorption, competes for same transporter), Fe (adequate iron ↓ Pb absorption), vitamin C. NHANES data: US children blood lead declining but racial disparities persist (higher in lower-income, older housing)
Arsenic	Inorganic arsenic (more toxic): groundwater contamination, rice (absorbs from flooded paddies — highest in brown rice), apple juice, food coloring. Organic arsenic (arsenobetaine): seafood — largely non-toxic and rapidly excreted. Chronic inorganic arsenic exposure: skin lesions, peripheral neuropathy, ↑ risk of skin, bladder, lung, kidney cancers (IARC Group 1). Infant rice cereal is a significant inorganic arsenic source → FDA limit 100 ppb in infant rice cereal. Counsel: diversify grains (oats, quinoa,

	barley, wheat) for rice-dominant diets; drain rice water after soaking (reduces arsenic ~28%)
Cadmium	Primary exposure: plant foods grown in Cd-contaminated soil (leafy vegetables, cereals, cocoa, potatoes), tobacco smoke, shellfish. Accumulates in kidney (cortex) → nephrotoxicity; bone damage (↑ fractures, osteoporosis — 'Itai-itai' disease in Japan). Half-life in kidney ~10–30 years — damage largely irreversible. Protective: adequate zinc, selenium, iron (compete with Cd at absorption; metallothionein binds Cd)
Dioxins & PCBs	Persistent Organic Pollutants (POPs). Industrial byproducts/contaminants. Bioaccumulate in fatty tissue → concentrate in animal fats (fatty fish, meat fat, dairy fat, eggs). IARC: TCDD (dioxin) Group 1 carcinogen; PCBs Group 1. Endocrine disruption (AhR activation), immunotoxicity, developmental neurotoxicity. Reduction: trim fat from meat and fish; moderate fatty fish consumption despite omega-3 benefits (risk-benefit analysis favours consumption for most — except high-exposure groups, pregnant women); prefer wild-caught Alaskan salmon and small fatty fish (lower PCBs than farmed Atlantic salmon historically)
Pesticide residues	EFSA annual monitoring: >97% of EU food samples have pesticide residues within MRLs. Organophosphates (most common pesticide class): acetylcholinesterase inhibitors — cumulative effects debated. 'Dirty Dozen' (EWG): strawberries, spinach, kale, peaches, pears — highest residues in US. Organic certification reduces (but doesn't eliminate) pesticide residue. Washing: 30-second cold water rinse removes some surface residues; removes no systemic pesticides. IARC: glyphosate (Roundup) Group 2A. Clinical perspective: health benefits of consuming conventional produce substantially exceed pesticide residue risks for most people — do not discourage fruit/vegetable consumption over pesticide concerns

IV. FOOD ALLERGIES & INTOLERANCES

A. Classification — Immunological Mechanisms

Adverse reactions to food must be carefully classified by underlying mechanism — this determines diagnosis, management, and prognosis:

Reaction Type	Mechanism	Key Examples
IgE-mediated food allergy (Type I hypersensitivity)	Allergen sensitization → IgE production (Th2/IL-4/IL-13 pathway) → IgE binds mast cells and basophils → re-exposure → crosslinking → mast cell degranulation → histamine, leukotrienes, prostaglandins released → immediate symptoms (minutes to 2 hours). Complement activation possible	The 'Big 9' allergens (US FASTER Act 2023): milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, soybeans, sesame. Most common cause of anaphylaxis. Peanuts: most common fatal food allergy reaction. Severity: urticaria/angioedema → GI symptoms → bronchospasm → cardiovascular collapse (anaphylaxis — BP drop, cardiovascular shock)
Non-IgE-mediated food allergy (Cell-mediated, Th1)	T-cell mediated delayed hypersensitivity; eosinophil involvement; mechanisms not fully characterized	FPIES (Food Protein-Induced Enterocolitis Syndrome): profuse vomiting 1–4h post-ingestion; rice, oats, cow's milk, soy in infants. Eosinophilic oesophagitis (EoE): eosinophil infiltration of oesophageal mucosa; milk, wheat, egg most common triggers. Coeliac disease (below)
Coeliac disease (Autoimmune, HLA-DQ2/DQ8)	Gluten (gliadin peptides) in genetically susceptible individuals (HLA-DQ2: 95%; DQ8: 5%) → adaptive immune response → anti-tTG (tissue transglutaminase) autoantibodies + T-cell-mediated small intestinal villous atrophy → malabsorption of all nutrients	Affects ~1% of population; majority undiagnosed. Consequences: iron/folate/Ca/fat-soluble vitamin malabsorption; infertility; ↑ osteoporosis; ↑ lymphoma risk. Diagnosis: anti-tTG IgA + endomysial antibody + duodenal biopsy (Marsh criteria). Treatment: lifelong strict gluten-free diet (wheat, rye, barley, contaminated oats). Oats: tolerated by ~80% of coeliac patients if certified gluten-free
Non-coeliac gluten sensitivity (NCGS)	Gluten triggers symptoms without autoimmune mechanism or IgE; innate immune pathway implicated; diagnosis by exclusion. FODMAPs in wheat may account for many NCGS cases	GI symptoms (bloating, diarrhoea, abdominal pain) + extraintestinal (fatigue, brain fog, headache). Negative coeliac serology and biopsy. Improve on gluten-free diet. Research active — mechanistic basis debated
Food intolerance (Non-immunological)	Enzymatic deficiency or pharmacological reaction — no immune mechanism involved	Lactose intolerance (lactase deficiency → undigested lactose → osmotic diarrhoea + fermentation gas). Fructose malabsorption. Histamine intolerance (DAO enzyme deficiency). Salicylate intolerance (COX pathway). Sulphite sensitivity (SO ₂ preservative → bronchoconstriction in asthma — IgE-independent)

B. Major Food Allergens — Nutritional Management

Allergen (Prevalence)	Hidden Sources & Nutritional Substitution Strategy
Cow's milk (~2–3% children; 0.1–0.5% adults)	Hidden in: processed meats, salad dressings, bread, margarine (casein/whey), dark chocolate. Label terms: casein, caseinates, whey, lactalbumin, lactoglobulin, ghee, hydrolyzed milk protein. Nutritional replacement: fortified plant-based milks (soy — best protein match, 7-8g/cup; oat, almond, coconut — lower protein unless enriched); calcium-set tofu; calcium-fortified orange juice; sardines with bones. Monitor: Ca ²⁺ , vitamin D, iodine (dairy is a major iodine source), riboflavin (B2), protein. Infants with IgE cow's milk allergy: extensively hydrolyzed casein formula (eHF) first choice; if refractory, amino acid-based formula
Peanuts (~1–2% all ages; rarely outgrown)	Hidden in: satay sauces, some chilli/curry products, baked goods, Asian cuisine, some chocolates, artificial nuts, cereal bars. Cross-contact risk in facilities processing tree nuts. Life-threatening anaphylaxis risk — require epinephrine auto-injector (EpiPen/AUVI-Q). PALFORZIA (peanut oral immunotherapy FDA-approved 2020 for ages 4–17) — desensitization; does not cure. Nutritional substitution: other nut/seed butters (sunflower seed, tahini) if tolerated; legume protein sources (lentils, chickpeas). ~20–25% of peanut allergic individuals also allergic to tree nuts
Tree nuts (~0.5–1%; rarely outgrown)	Hidden in: pestos (pine nuts, walnuts), praline, marzipan (almond), cereals, chocolate, plant milks, natural flavourings. Individual tree nuts (almond, cashew, walnut, pecan, pistachio, Brazil nut, hazelnut, macadamia) are antigenically distinct — allergy to one does not mandate avoidance of all (but cross-reactivity common). Avoidance tailored to specific reactivity by allergy testing
Wheat (~0.5–1%; often outgrown in children)	Hidden in: soy sauce (traditional — contains wheat), kamut, spelt, farro, durum, semolina, some oats, modified food starch, hydrolyzed vegetable protein. Distinct from coeliac disease (IgE mechanism, normal bowel histology). Nutritional replacement: rice, oats (if not cross-contaminated), quinoa, millet, buckwheat, corn, potato, cassava flours. Monitor: B vitamins (if avoiding fortified wheat products), fibre (refined GF products often lower fibre than wheat equivalents)
Shellfish (~2% adults; often lifelong)	Hidden in: Worcestershire sauce (anchovies — actually fish), seafood stocks, sauces, risottos, paella, glucosamine supplements (shellfish-derived). Shell (crustacean) allergy (shrimp, crab, lobster, crayfish) and mollusc allergy (clams, oysters, scallops, squid) are distinct IgE profiles — cross-reactivity variable. Tropomyosin is major allergen in crustaceans and molluscs. Nutritional: omega-3 supplementation (algal DHA) to replace seafood omega-3s; iodine supplement (seafood is major dietary iodine source)
Egg (~2% children; ~65% outgrow by adolescence)	Hidden in: pasta (fresh), baked goods, mayonnaise, aioli, hollandaise, meringue, nougat, glazes (egg wash), processed meats, foam in cocktails, some vaccines (influenza — grown in eggs; MMR-contraindicated if severe egg allergy, discuss with allergist). Egg white (albumin, ovomucoid) more allergenic than yolk. Cooking increases egg tolerance for many (baked egg tolerance in 70% of egg-allergic children). Nutritional: egg protein is high DIAAS reference protein; substitute: legumes, dairy/meat for protein; chia/flax 'eggs' (1 tbsp ground + 3 tbsp water) as baking substitute; choline from other sources (liver, soy, fish)

Soy (~0.4% children; often outgrown)	Hidden in: edamame, tofu, tempeh, miso, soy sauce, many processed foods (lecithin, hydrolyzed vegetable/plant protein, textured vegetable protein). Soy lecithin: typically tolerated even by soy-allergic individuals (low protein content). Soy formula: generally safe for cow's milk-allergic infants over 6 months; not suitable for very young infants with FPIES to cow's milk. Monitor: if avoiding soy, replace plant protein with other legumes/meat/dairy; ensure iodine intake (soy contains goitrogens)
Sesame (Added as 9th major allergen — FASTER Act 2023)	Hidden in: tahini, hummus, halva, bakery products (sesame seed topping), some Middle Eastern, Asian, and Mediterranean dishes, some supplements and medications. Prevalence: ~0.2–0.5%; severity: can be severe anaphylaxis. Previously unlabelled in US — FASTER Act mandates 'sesame' labelling from 2023. Cross-reactivity with other seeds (poppy, kiwi) possible. Nutritional note: sesame is a significant source of calcium (tahini: 130 mg/2 tbsp) and zinc

C. Lactose Intolerance — A Distinct Clinical Entity

Lactose intolerance (LI) is not a food allergy but the most common food intolerance globally (estimated 65–70% of world adult population), resulting from reduced or absent lactase enzyme (LCT gene expression declines with age — 'lactase non-persistence'). Prevalence highly variable by ethnicity: Southeast Asian/East Asian ~95%; Sub-Saharan African ~75%; Hispanic ~53%; White Northern European ~5–15%.

- Mechanism: Undigested lactose → osmotic diarrhoea (draws water into lumen) + colonic bacterial fermentation → SCFAs + H₂, CH₄, CO₂ gases → bloating, flatulence, abdominal cramping. Symptoms dose-dependent — most individuals tolerate 12g lactose (1 cup milk) without symptoms, especially with food. Threshold varies by individual and colonic microbiome composition
- Diagnosis: Hydrogen breath test (gold standard — measure H₂ in expired air after 50g lactose challenge; >20 ppm rise = positive); lactose tolerance blood test (BG rise <20 mg/dL = malabsorption); genetic testing (LCT gene C/T-13910 polymorphism — identifies lactase persistence)
- Management: Most LI individuals do NOT need to eliminate dairy entirely — lactose reduction strategies: (1) Consume dairy with food; (2) Choose aged/hard cheeses (low lactose — <1g/serving); (3) Choose yogurt (Lactobacillus produces lactase during fermentation); (4) Lactase enzyme supplements (Lactaid) with meals; (5) Lactose-free dairy products (lactase pre-treated); (6) Gradually increase dairy intake — colonic microbiome adapts; (7) Cultured milk/kefir better tolerated than pasteurized milk

Clinical Note: Lactose intolerance vs cow's milk allergy: Frequently confused by patients. LI is dose-dependent, non-immunological, and symptoms limited to GI (bloating, diarrhoea, gas — within 30 min to 2 hours). Cow's milk allergy (IgE-mediated) can cause anaphylaxis, urticaria, angioedema — even trace exposure. Misdiagnosis of cow's milk allergy as LI is dangerous. Proper allergy testing (skin prick, specific IgE, oral food challenge under supervision) essential to distinguish.

Lecture 3 Summary — Key Principles

- Farm-to-fork: nutritional vulnerability exists at every stage — soil depletion (selenium, iodine, zinc), post-harvest losses (vitamin C, carotenoids), processing losses (folate, thiamin, vitamin C), and contamination (aflatoxins, pesticides, heavy metals). Fortification (mandatory) is the most cost-effective global intervention
- Thermal processing: vitamin C and folate are most labile; fat-soluble vitamins are relatively stable; Maillard reaction → AGEs + ↓ lysine bioavailability; resistant starch increases on cooling (beneficial); omega-3 oxidation in repeated deep-frying
- NOVA classification: Group 4 ultra-processed foods are independently associated with CVD, T2DM, cancer, and all-cause mortality; mechanisms include hyper-palatability, fibre/phytonutrient displacement, food matrix disruption, and additives
- Natural toxicants: lectins (cooking destroys), oxalates (bind minerals, kidney stones), phytate (mineral chelation — reduces with soaking/fermentation), glycoalkaloids (potatoes — discard green), cyanogenic glycosides (cassava — proper processing), goitrogens (significant only in iodine deficiency)
- Processing-derived toxicants: acrylamide (high-temp starchy foods — IARC 2A), HCAs/PAHs (grilled/barbecued meats — IARC 1), trans FA (largely eliminated post-FDA ban), nitrosamines (processed meats — WCRF colorectal cancer link), AGEs (high-heat animal products)
- Food allergy classification: IgE-mediated (rapid, anaphylaxis risk — Big 9 allergens), non-IgE cell-mediated (FPIES, EoE), autoimmune (coeliac — gluten, HLA-DQ2/DQ8, villous atrophy), and non-immune intolerance (lactose — enzymatic, dose-dependent, not life-threatening)
- Nutritional management of food allergies requires systematic replacement of excluded food groups — calcium/B12/iodine/omega-3 when dairy excluded; B12/DHA/iodine/iron/zinc/calcium when plant-based diet adopted

Suggested Reading:

1. Codex Alimentarius (2023). Food Safety and Contaminants Standards. |
2. Monteiro CA et al. (2019). Ultra-processed foods: what they are and how to identify them. Public Health Nutr. |
3. Matricardi PM et al. (2016). EAACI molecular allergology user's guide. Pediatr Allergy Immunol.
4. WHO/FAO (2004). Vitamin and Mineral Requirements in Human Nutrition, 2nd Ed. |
5. IARC Monographs Vol. 114 (2015). Red meat and processed meat.

Module 4

Oxidative Stress, Phytochemicals & Functional Foods

Professor Osama Awad Salih

NUTRITION & DIETETICS

MS Program Lecture Series

Oxidative Stress, Phytochemicals & Functional Foods

A 3-Lecture Graduate Series

Free Radicals & Antioxidants • Phytochemicals • Fruits & Vegetables

Professor Osama Awad Salih

Lecture 1

Free Radicals &
Antioxidant Defense

Lecture 2

Phytochemicals:
Sources & Benefits

Lecture 3

Fruits & Vegetables:
Micronutrient Powerhouses

LECTURE 1

Free Radicals, Oxidative Damage & Antioxidant Defense

Biochemical Mechanisms, Chronic Disease Pathways & Therapeutic Strategies

Nutrition & Dietetics MS Program | Department of Nutritional Sciences

Learning Outcomes — By the end of this lecture, students will be able to:

- Define free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) and classify them by reactivity
- Describe the four major cellular damage mechanisms: lipid peroxidation, protein oxidation, DNA oxidation, and glycoxidation
- Explain the enzymatic and non-enzymatic antioxidant defense systems and their interactions
- Connect oxidative stress to the pathophysiology of CVD, cancer, and type 2 diabetes
- Evaluate the clinical and epidemiological evidence for dietary antioxidants in chronic disease prevention
- Apply oxidative stress concepts to practical dietary counseling strategies

I. FOUNDATIONS OF FREE RADICAL BIOLOGY

A. Definitions & Terminology

A free radical is any atom, molecule, or ion with one or more unpaired electrons in its outer orbital. This unpaired electron confers high chemical reactivity — free radicals avidly extract electrons from neighboring molecules, generating new radicals in a chain reaction. Reactive oxygen species (ROS) is a broader term encompassing both radical and non-radical oxidants derived from oxygen.

Species	Classification & Key Properties
Superoxide anion ($O_2^{\bullet-}$)	Radical; primary ROS from mitochondrial electron leak (Complex I & III) and NADPH oxidase; relatively mild oxidant but precursor to more reactive species; dismutated by SOD
Hydrogen peroxide (H_2O_2)	Non-radical ROS; produced by SOD from $O_2^{\bullet-}$; diffuses across membranes; signaling molecule at low concentrations; precursor to $\bullet OH$ via Fenton reaction
Hydroxyl radical ($\bullet OH$)	Radical; most reactive biological ROS ($t_{1/2} \sim 10^{-9}$ s); produced by Fenton ($Fe^{2+} + H_2O_2$) and Haber-Weiss reactions; reacts at site of formation; no enzyme eliminates it
Singlet oxygen (1O_2)	Non-radical excited O_2 ; produced by UV radiation and myeloperoxidase; damages lipids and DNA; quenched by carotenoids

Peroxynitrite (ONOO ⁻)	Non-radical RNS; from O ₂ • ⁻ + NO•; highly reactive; nitrates tyrosine residues; extremely damaging to proteins and DNA
Nitric oxide (NO•)	Radical RNS; produced by NOS enzymes; signaling (vasodilation, neurotransmission); becomes harmful when combines with O ₂ • ⁻
Hypochlorous acid (HOCl)	Non-radical; from H ₂ O ₂ + Cl ⁻ via myeloperoxidase (MPO) in neutrophils; potent antimicrobial; damages proteins and lipids during chronic inflammation

B. Sources of ROS — Endogenous & Exogenous

Endogenous Sources:

- Mitochondrial electron transport chain (ETC): Major endogenous source. ~0.2–2% of O₂ consumed is converted to O₂•⁻ at Complex I (NADH dehydrogenase) and Complex III (ubiquinol-cytochrome c reductase). Electron leak is proportional to membrane potential — higher mitochondrial activity = more ROS
- NADPH oxidase (NOX) family: 7 isoforms (NOX1–5, DUOX1–2). NOX2 in phagocytes produces deliberate O₂•⁻ as part of the respiratory burst (antimicrobial defense). NOX4 in vascular cells produces H₂O₂ for redox signaling. Dysregulated NOX activity → chronic oxidative stress
- Xanthine oxidase: Purine catabolism (hypoxanthine → xanthine → uric acid) generates O₂•⁻ and H₂O₂; activated during ischemia-reperfusion injury
- Cytochrome P450 enzymes: Phase I drug metabolism in liver ER; electron uncoupling generates ROS; elevated with alcohol consumption, drug metabolism, obesogenic diet
- Peroxisomes: H₂O₂ generated by fatty acid β-oxidation; normally degraded by catalase within the organelle
- Transition metal catalysis: Free Fe²⁺ and Cu⁺ catalyze Fenton reaction → •OH from H₂O₂; iron dysregulation (hemochromatosis, repeated transfusions) amplifies oxidative damage

Exogenous Sources:

- Diet: Oxidized lipids (fried foods, rancid oils), advanced glycation end products (AGEs), dietary pro-oxidants
- Environmental: UV radiation (skin ROS → skin cancer), ionizing radiation, air pollution (PM2.5 — generates ROS directly and induces NOX), heavy metals (Pb, Hg, Cd, As — deplete GSH, displace catalytic metals)
- Lifestyle: Cigarette smoke (~10¹⁵ free radicals per puff; includes O₂•⁻, NO•, H₂O₂, acrolein); alcohol (CYP2E1-mediated ROS, acetaldehyde); physical overtraining (skeletal muscle ROS from intense contractions)

II. MECHANISMS OF OXIDATIVE DAMAGE

A. Lipid Peroxidation

Lipid peroxidation (LPO) is the oxidative degradation of polyunsaturated fatty acids (PUFAs) in cell membranes and lipoproteins. It proceeds as a chain reaction in three stages:

- Initiation: $\bullet\text{OH}$ (or $^1\text{O}_2$) abstracts a bisallylic hydrogen from a PUFA $\rightarrow$ carbon-centered lipid radical ($\text{L}\bullet$); particularly susceptible PUFAs: arachidonic acid (4 double bonds), EPA (5), DHA (6)
- Propagation: $\text{L}\bullet + \text{O}_2 \rightarrow$ lipid peroxy radical ($\text{LOO}\bullet$) $\rightarrow$ $\text{LOO}\bullet$ abstracts H from adjacent PUFA $\rightarrow$ lipid hydroperoxide (LOOH) + new $\text{L}\bullet$; each cycle produces a new radical — chain reaction amplifies damage
- Termination: Two radicals combine to form non-radical products; OR chain-breaking antioxidants (vitamin E, ubiquinol) donate $\text{H}\bullet$ to $\text{LOO}\bullet \rightarrow \text{LOOH} + \text{Toc}\bullet$ ($\text{Toc}\bullet$ regenerated by vitamin C)

Key Lipid Peroxidation Products & Their Significance:

Product	Significance & Use as Biomarker
Malondialdehyde (MDA)	Most studied; measured by TBARS assay; reacts with DNA bases (etheno-adducts) and proteins (cross-links); urinary MDA used in clinical research
4-Hydroxynonenal (4-HNE)	Highly reactive aldehyde from ω -6 PUFA peroxidation; forms protein and DNA adducts; activates Nrf2 at low doses; toxic at high doses; plaque formation, hepatotoxicity
Acrolein	From ω -3 and ω -6 PUFAs; extremely reactive; DNA adducts (propano-dGuo); major contributor to cigarette smoke toxicity
F2-isoprostanes (8-iso-PGF2 α)	Gold standard biomarker of in vivo lipid peroxidation; produced non-enzymatically from arachidonic acid; stable, measurable in urine and plasma; elevated in CVD, T2DM, smoking, obesity
Oxidized LDL (oxLDL)	LOOH-modified ApoB-100; recognized by scavenger receptors on macrophages $\rightarrow$ foam cells $\rightarrow$ atherosclerotic plaque; central to CVD pathogenesis

B. Protein Oxidation

Proteins are major targets of ROS, particularly $\bullet\text{OH}$ and ONOO^- . Oxidative protein modifications include:

- Carbonylation: Oxidation of Pro, Arg, Lys, Thr residues $\rightarrow$ carbonyl groups (aldehydes/ketones); carbonyl content is the most widely used marker of protein oxidative damage; irreversible; accumulates with aging and chronic disease
- Sulfenic/sulfinic/sulfonic acid formation: Oxidation of cysteine thiol ($-\text{SH}$) $\rightarrow$ sulfenic acid ($-\text{SOH}$, reversible) $\rightarrow$ sulfinic ($-\text{SO}_2\text{H}$, largely irreversible) $\rightarrow$ sulfonic acid ($-\text{SO}_3\text{H}$, irreversible); disrupts enzyme active sites and structural disulfide bonds
- Tyrosine nitration: ONOO^- nitrates tyrosine $\rightarrow$ 3-nitrotyrosine (3-NT); disrupts phosphorylation signaling; biomarker of nitrosative stress; elevated in atherosclerosis, ALS, Alzheimer's
- Methionine oxidation: Met $\rightarrow$ methionine sulfoxide; reversed by methionine sulfoxide reductase (MSRA/B) — requires thioredoxin; protective mechanism with high repair capacity

- Cross-linking & aggregation: Oxidized proteins form insoluble aggregates (dityrosine cross-links); contributes to lens cataracts, Alzheimer's plaques, Parkinson's Lewy bodies

Clinical Note: *Proteasomal degradation normally removes oxidized proteins, but chronic severe oxidative stress overwhelms this system, leading to accumulation of damaged protein aggregates — a hallmark of neurodegenerative diseases and cellular aging.*

C. DNA Oxidation

DNA is continuously damaged by endogenous ROS — estimated 10,000–100,000 oxidative DNA lesions per cell per day, repaired primarily by base excision repair (BER). Persistent unrepaired lesions cause mutations and contribute to carcinogenesis.

- 8-Oxo-7,8-dihydro-2'-deoxyguanosine (8-OHdG / 8-oxodG): Most common and well-studied oxidative DNA lesion; •OH attack at C8 of guanine; mispairs with adenine during replication → G:C to T:A transversion mutation; a hotspot in proto-oncogenes (e.g., codon 12 of KRAS). Urinary 8-OHdG is a validated biomarker of systemic oxidative DNA damage; elevated in cancer, T2DM, CVD, obesity
- Formamidopyrimidines (FapyG, FapyA): Ring-opened purine lesions; highly mutagenic; repaired by OGG1 glycosylase
- Strand breaks: •OH can cleave the deoxyribose sugar-phosphate backbone → single-strand breaks (SSBs) or double-strand breaks (DSBs); DSBs are the most cytotoxic; repaired by HR and NHEJ pathways
- Base loss (abasic/AP sites): ROS-induced depurination or depyrimidination → unstable AP site → strand break if unrepaired
- DNA-protein crosslinks: Highly cytotoxic; block replication and transcription; induced by •OH and aldehydes (MDA, 4-HNE)

Clinical Note: *Antioxidant enzyme polymorphisms (OGG1 Ser326Cys, MnSOD Val16Ala, CAT -262C>T) are associated with elevated cancer risk in population studies — evidence that individual antioxidant defense capacity modulates cancer susceptibility.*

D. Glycooxidation & Advanced Glycation End Products (AGEs)

Glycooxidation occurs when reducing sugars react non-enzymatically with amino groups of proteins, lipids, and DNA (Maillard reaction / Amadori rearrangement) → Schiff base → Amadori product → further oxidation → Advanced Glycation End Products (AGEs). Accelerated by hyperglycemia and oxidative stress (hence 'glyco-oxidation').

- Endogenous AGEs: Carboxymethyllysine (CML), pentosidine, methylglyoxal (MGO)-derived adducts; accumulate in long-lived proteins (collagen, elastin, myelin, lens crystalline)
- Exogenous dietary AGEs: Generated during high-heat cooking (grilling, frying, baking at >150°C); estimated 10–40% absorbed; contribute to systemic AGE pool
- RAGE (Receptor for AGEs): Pattern recognition receptor on macrophages, endothelial cells, neurons; AGE-RAGE binding → NF-κB activation → inflammatory cytokines (TNF-α, IL-1β, IL-6) → perpetuates oxidative-inflammatory cycle

Evidence: *In T2DM, elevated HbA1c directly reflects Amadori product formation on hemoglobin. Serum AGEs and sRAGE (soluble RAGE, decoy receptor) are emerging biomarkers of glycooxidative burden and CVD/diabetic complication risk.*

Counseling Tip: *Advise patients to favor lower-temperature cooking methods (steaming, poaching, stewing) over grilling/frying to reduce dietary AGE intake. Marinating meat with acidic ingredients (lemon, vinegar) before grilling reduces AGE formation by ~50%.*

III. ANTIOXIDANT DEFENSE SYSTEMS

A. The Antioxidant Network — Overview

The antioxidant defense system operates as an integrated, hierarchical network. No single antioxidant works in isolation — they function cooperatively, with each component protecting or regenerating others. The network has two major arms: enzymatic (primary, catalytic, highly efficient) and non-enzymatic (dietary, regenerable, lipid- and water-phase coverage).

B. Enzymatic Antioxidant Defense — First Line

Enzyme (Gene)	Reaction & Compartment Cofactors & Clinical Notes
Superoxide dismutase 1 (SOD1 — Cu/Zn-SOD)	$2\text{O}_2^{\bullet-} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$ Cytosol; requires Cu^{2+} and Zn^{2+} ; Cu deficiency ↓ activity; SOD1 mutations cause familial ALS
Superoxide dismutase 2 (SOD2 — MnSOD)	Same reaction Mitochondrial matrix; requires Mn^{2+} ; critical at primary ROS source; Val16Ala polymorphism ↑ cancer risk; ↑ by fasting, caloric restriction
Superoxide dismutase 3 (SOD3 — EC-SOD)	Same reaction Extracellular; protects vascular endothelium; released by exercise; heparin-binding to ECM
Catalase (CAT)	$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$ Peroxisomes, erythrocytes; iron (heme) cofactor; extremely high rate ($k \sim 10^7 \text{ M}^{-1}\text{s}^{-1}$); -262C>T promoter SNP ↓ expression; reduced in metabolic syndrome
Glutathione peroxidase 1 (GPx1)	$\text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{GSSG} + 2\text{H}_2\text{O}$ Cytosol + mitochondria; selenocysteine active site; selenium-dependent; P200L polymorphism ↑ CVD & cancer risk
GPx4 (Phospholipid-GPx)	$\text{LOOH in membranes} \rightarrow \text{LOH}$ Only enzyme that reduces membrane phospholipid hydroperoxides in situ; key ferroptosis regulator; Se-dependent; essential for spermatogenesis
Glutathione reductase (GR)	$\text{GSSG} + \text{NADPH} \rightarrow 2\text{GSH}$ Regenerates GSH from GSSG; FAD (B2)-dependent; NADPH from pentose phosphate pathway; links riboflavin and niacin to antioxidant defense
Thioredoxin system (TrxR / Trx)	$\text{Trx-S}_2 + \text{NADPH} \rightarrow \text{Trx-(SH)}_2$ Reduces oxidized proteins; selenium + FAD cofactors; regenerates Prx, ribonucleotide reductase, ascorbate; auranofin (TrxR inhibitor used in cancer therapy)
Peroxiredoxins (Prx1–6)	$\text{H}_2\text{O}_2 / \text{ROOH} \rightarrow \text{H}_2\text{O} / \text{ROH}$ Ubiquitous; reduce H_2O_2 , organic peroxides, peroxynitrite; Prx3 (mitochondrial), Prx6 (phospholipid peroxide); major H_2O_2 scavengers at low concentrations
Heme oxygenase-1 (HO-1 / HMOX1)	$\text{Heme} \rightarrow \text{biliverdin} + \text{CO} + \text{Fe}^{2+}$ Inducible by Nrf2, oxidative stress, heme; cytoprotective products: biliverdin/bilirubin (antioxidant), CO (anti-inflammatory vasodilator); Fe^{2+} bound by ferritin

C. Glutathione (GSH) — The Master Intracellular Antioxidant

Glutathione (L-γ-glutamyl-L-cysteinylglycine) is the most abundant intracellular thiol antioxidant (~1–10 mM intracellularly). The ratio GSH:GSSG reflects cellular redox status — healthy cells maintain >100:1 (GSH:GSSG); oxidative stress shifts this ratio.

- Synthesis: Two-step ATP-dependent process: (1) Glutamate + cysteine → γ-glutamylcysteine [via GCL — rate-limiting; product of Nrf2 activation]; (2) γ-glutamylcysteine + glycine → GSH [via GSS]
- Cysteine availability is the rate-limiting substrate for GSH synthesis — N-acetylcysteine (NAC) is a cysteine prodrug used clinically to restore GSH (acetaminophen toxicity, chronic lung disease, contrast nephropathy)
- Functions: (1) GPx substrate for H₂O₂ and LOOH reduction; (2) S-glutathionylation — reversible protein cysteine modification for redox signaling; (3) GST substrate — conjugation of electrophiles/xenobiotics for Phase II detoxification; (4) Transport of amino acids (γ-glutamyl cycle)
- GSH depletion in disease: Severe in HIV/AIDS, COPD, liver disease, acetaminophen toxicity, alcoholic hepatitis; correlates with disease severity and mortality in critical illness

D. The Nrf2/Keap1 Pathway — Master Regulator of Antioxidant Gene Expression

Nrf2 (Nuclear factor erythroid 2-related factor 2) is the master transcription factor for the cellular antioxidant and electrophile stress response. Under basal conditions, Nrf2 is bound to Keap1 (Kelch ECH-associating protein 1) in the cytoplasm and targeted for proteasomal degradation. Under oxidative or electrophilic stress, Keap1 sensor cysteines (Cys151, Cys273, Cys288) are modified → conformational change → Nrf2 escapes degradation → translocates to nucleus → binds Antioxidant Response Elements (ARE) in gene promoters.

Nrf2 target genes (>200 identified):

- Antioxidant enzymes: GCLC/GCLM (GSH synthesis), HO-1, NQO1, GPx2, TrxR1, PRDX1
- Phase II detoxification: GSTs (α,μ,π), UGTs, SULTs, ALDH — conjugate and eliminate carcinogens and xenobiotics
- Other cytoprotective: Ferritin (sequesters redox-active Fe), biliverdin reductase, proteasome subunits

Evidence: *Sulforaphane (from broccoli) is the most potent known dietary Nrf2 activator — it modifies Keap1 Cys151 via Michael addition. Other dietary Nrf2 activators: curcumin, resveratrol, quercetin, EGCG, allicin, lycopene. This mechanism underpins the chemo-preventive properties of many plant foods.*

E. Non-Enzymatic Dietary Antioxidants

Antioxidant	Mechanism of Action & Key Notes
α -Tocopherol (Vitamin E)	Chain-breaking lipid antioxidant; donates H• to $\text{LOO}^\bullet \rightarrow \text{LOOH} + \text{Toc}^\bullet$; $\text{Toc}^\bullet$ regenerated by ascorbate; protects membrane PUFAs and LDL; main lipid-phase antioxidant
Ascorbic Acid (Vitamin C)	Primary aqueous-phase antioxidant; scavenges $\text{O}_2^{\bullet-}$, $\bullet\text{OH}$, HOCl , $^1\text{O}_2$; regenerates α -tocopherol at membrane-aqueous interface; reduces $\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$ (pro-oxidant at pharmacological doses in presence of free Fe)
β -Carotene & Carotenoids	Singlet oxygen ($^1\text{O}_2$) quenching (physical and chemical); radical scavenging; pro-oxidant at high doses in hypoxia (explains CARET trial findings in smokers)
Polyphenols	Indirect antioxidants via Nrf2 activation; direct radical scavenging ($\text{H}^\bullet$ donation via ArOH); metal chelation (Fe , $\text{Cu} \rightarrow$ prevents Fenton reaction); structural catechol/ortho-dihydroxy groups confer highest activity
Glutathione (GSH)	Primary intracellular non-enzymatic thiol antioxidant; GPx cosubstrate; sacrificial scavenger of $\bullet\text{OH}$ and HOCl ; poor oral bioavailability — NAC, cysteine-rich foods more effective
Uric Acid	Major antioxidant in human plasma (evolutionary loss of uricase); scavenges $\bullet\text{OH}$, ONOO^- , HOCl ; protects ascorbate; paradoxically pro-inflammatory via NLRP3 inflammasome at intracellular level
Bilirubin	Potent lipid antioxidant; chain-breaking properties; mild hyperbilirubinemia (Gilbert's syndrome) inversely associated with CVD and cancer risk in epidemiological studies
Ubiquinol (CoQ10)	Reduced form of ubiquinone; membrane lipid antioxidant; regenerates vitamin E; found in inner mitochondrial membrane; synthesized endogenously (declines with aging, statins deplete)

IV. OXIDATIVE STRESS IN CHRONIC DISEASE

A. Cardiovascular Disease (CVD)

Oxidative stress is mechanistically central to all stages of atherosclerosis — from endothelial dysfunction through plaque rupture:

- Endothelial dysfunction: $\text{O}_2^{\bullet-}$ uncouples eNOS (converts BH_4 to $\text{BH}_2 \rightarrow$ eNOS produces $\text{O}_2^{\bullet-}$ instead of $\text{NO}^\bullet$) AND $\text{O}_2^{\bullet-}$ rapidly reacts with $\text{NO}^\bullet \rightarrow \text{ONOO}^- \rightarrow$ loss of vasodilatory NO, gain of oxidant damage; endothelial dysfunction measured as impaired flow-mediated dilation (FMD)
- LDL oxidation: Subendothelial LDL undergoes lipid peroxidation ($\text{LOO}^\bullet$ chain reaction on ApoB-100-associated PUFAs) $\rightarrow$ oxLDL; recognized by SR-A and CD36 scavenger receptors on macrophages $\rightarrow$ unregulated uptake $\rightarrow$ foam cells $\rightarrow$ fatty streak $\rightarrow$ plaque core
- NF- κB activation: ROS (particularly H_2O_2 from NOX2/4) activate NF- $\kappa\text{B} \rightarrow \uparrow$ VCAM-1, ICAM-1, MCP-1 $\rightarrow$ monocyte recruitment and adhesion $\rightarrow$ plaque progression
- Plaque instability and rupture: Oxidized lipids and proteases from macrophages degrade fibrous cap $\rightarrow$ plaque rupture $\rightarrow$ thrombosis $\rightarrow$ MI/stroke

Evidence: *The Mediterranean diet (PREDIMED trial, n=7447): 30% ↓ major cardiovascular events. Associated with lower F2-isoprostane and oxLDL levels. Diets rich in polyphenols, MUFAs, omega-3s, and vitamin E reduce biomarkers of lipid peroxidation and endothelial dysfunction.*

B. Cancer

Oxidative stress drives carcinogenesis at multiple stages:

- Initiation: •OH-induced 8-OHdG lesions in proto-oncogenes and tumor suppressor genes (TP53, KRAS, APC) → if unrepaired before replication → permanent mutations
- Promotion: ROS activate NF-κB and AP-1 transcription factors → ↑ inflammatory mediators (COX-2, TNF-α) + survival signals (Bcl-2, c-Myc) → clonal expansion of initiated cells
- Progression: Sustained oxidative stress → genomic instability (impaired mismatch repair, telomere dysfunction) → malignant progression; high ROS in tumor microenvironment promotes angiogenesis (HIF-1α stabilized by prolyl hydroxylase inhibition)

Clinical Note: *Paradox: While dietary antioxidants may reduce cancer initiation/promotion, established tumors exploit ROS for growth signaling. High-dose antioxidant supplementation during cancer treatment may blunt chemo/radiotherapy efficacy (which kills cancer cells partly via ROS) — an important clinical counseling consideration.*

Evidence: *Prospective cohort meta-analyses: Higher dietary antioxidant scores (DASI, ORAC-based) inversely associated with colorectal cancer, lung cancer, and upper GI cancers. Supplemental antioxidants show weaker or null effects — supporting whole food sources.*

C. Type 2 Diabetes (T2DM)

A bidirectional, reinforcing relationship exists between hyperglycemia and oxidative stress:

- Hyperglycemia → ROS: (1) Glucose autooxidation → $O_2^{\bullet-}$; (2) Polyol pathway activation (aldose reductase) → consumes NADPH (needed for GSH regeneration) → oxidative stress; (3) PKC activation by DAG → NOX activation → $O_2^{\bullet-}$; (4) Mitochondrial superoxide overproduction from excess electron donors (NADH, $FADH_2$)
- ROS → Insulin resistance: H_2O_2 and 4-HNE impair insulin receptor substrate (IRS-1) signaling via serine phosphorylation (activated by JNK, IKK) → uncouples insulin receptor from PI3K/Akt pathway → ↓ GLUT4 translocation → insulin resistance
- Beta-cell vulnerability: Pancreatic β-cells express very low catalase and GPx1 relative to other tissues → particularly susceptible to H_2O_2 -mediated damage → β-cell apoptosis → progressive insulin secretory failure
- AGE-RAGE axis: Glycoxidation of proteins (as above) activates RAGE → NF-κB → inflammatory cytokines → further insulin resistance and endothelial dysfunction

Evidence: *Biomarkers of oxidative stress (8-OHdG, F2-isoprostanes, MDA) are elevated in pre-diabetes and T2DM patients and predict complication development. Dietary patterns rich in antioxidants (Mediterranean, DASH) reduce incident T2DM risk by 20-30% in prospective cohorts.*

V. DIETARY ANTIOXIDANTS: CLINICAL EVIDENCE & COUNSELING

A. The Supplement Paradox — Why Food Beats Pills

Despite compelling mechanistic data, large randomized controlled trials of antioxidant supplements have been largely disappointing or even harmful:

Trial	Intervention & Outcome
ATBC (Finland, 1994)	Alpha-tocopherol + β -carotene in male smokers $\rightarrow$ $\uparrow$ 18% lung cancer in β -carotene arm (reversed expected protection)
CARET (USA, 1996)	Beta-carotene + retinyl palmitate in smokers/asbestos workers $\rightarrow$ trial stopped early: $\uparrow$ 28% lung cancer, $\uparrow$ 17% CVD mortality
HOPE (2000)	Vitamin E 400 IU/day in high-CVD-risk patients $\rightarrow$ no benefit; HOPE-TOO extension: $\uparrow$ heart failure risk
SELECT (2011)	Vitamin E + selenium for prostate cancer prevention $\rightarrow$ Vitamin E $\uparrow$ prostate cancer by 17% in selenium-adequate men
Heart Protection Study	Vitamin E + C + β -carotene $\rightarrow$ no reduction in vascular events, cancer, or mortality despite $\downarrow$ LDL oxidation markers
AREDS2 (AMD)	Lutein 10mg + zeaxanthin 2mg $\rightarrow$ $\downarrow$ 25% progression to advanced AMD; replaced β -carotene — one of few positive supplement RCTs

Explanations for supplement failures:

- Antioxidant network disruption — isolated high-dose supplements displace endogenous antioxidants and distort the physiological balance;
- Pro-oxidant effects — at pharmacological doses, ascorbate (in presence of free Fe) and β -carotene (in high PO_2 in smokers' lungs) can generate ROS;
- Hormetic signaling disruption — low-dose ROS function as essential signaling molecules (e.g., exercise-induced ROS activates PGC-1 α for mitochondrial biogenesis; high-dose antioxidants blunt this adaptation);
- Food matrix and synergy — whole foods provide antioxidants alongside fiber, prebiotics, and hundreds of co-occurring phytonutrients with synergistic effects.

B. Measuring Antioxidant Capacity — Tools & Limitations

Assay	Principle & Limitations
ORAC (Oxygen Radical Absorbance Capacity)	Measures H• transfer capacity; food labeling gold standard (withdrawn by USDA 2012 as poor predictor of in vivo effects)
FRAP (Ferric Reducing Antioxidant Power)	Electron transfer to Fe ³⁺ ; quick, cheap; measures only aqueous phase; does not measure chain-breaking activity
DPPH Radical Scavenging	Colorimetric; simple; measures H• and electron transfer; poor physiological relevance
Serum TAS (Total Antioxidant Status)	Plasma antioxidant capacity; dominated by uric acid (~60%); limited clinical utility as standalone marker
F2-isoprostanes (urine/plasma)	Gold standard in vivo oxidative stress biomarker; not affected by diet composition; reflects actual lipid peroxidation
8-OHdG (urine)	Validated DNA oxidation biomarker; reflects oxidative DNA damage and repair; used in carcinogenesis research
MDA / TBARS	Simple, widely used; lacks specificity; falsely elevated by methodology artifacts; use with caution

C. Practical Dietary Counseling — Antioxidant Defense Optimization

Counseling Tip: *Emphasize total dietary pattern over individual nutrients. The 'antioxidant score' of a diet is more predictive of health outcomes than any single antioxidant intake.*

- Maximize plant food diversity: Each plant food contains unique combination of phytonutrients — 'eat the rainbow' maximizes antioxidant network coverage
- Priority foods: Berries (anthocyanins), cruciferous vegetables (sulforaphane/Nrf2), citrus (vitamin C, flavanones), nuts (vitamin E, polyphenols), extra virgin olive oil (oleocanthal, hydroxytyrosol), dark leafy greens (lutein, zeaxanthin, vitamins C & E), legumes (GSH precursors, polyphenols)
- Cooking considerations: Boiling leaches water-soluble antioxidants (vitamin C); steaming and stir-frying preserve better; tomato cooking ↑ lycopene bioavailability (disrupts cell walls); β-carotene absorption enhanced by fat co-ingestion
- Limit pro-oxidant exposures: Minimize processed meats (nitrosamines, heme iron-driven peroxidation), refined carbohydrates (hyperglycemia-driven ROS), oxidized oils (high-heat repeated frying), tobacco, excess alcohol
- Micronutrient adequacy: Ensure selenium (soil content varies; Brazil nuts, seafood), zinc (immune/SOD function), copper (SOD1/3), riboflavin (GR regeneration), and vitamin D (anti-inflammatory) — deficiencies impair enzymatic antioxidant defense
- Targeted supplementation: Reserve for verified deficiency or specific clinical indication (e.g., vitamin E in NAFLD: some evidence for 800 IU/day in non-diabetic NASH; lutein/zeaxanthin in AMD; NAC in acetaminophen toxicity, contrast nephropathy prevention)

Lecture 1 Summary — Key Principles

- Free radicals ($O_2^{\bullet-}$, $\bullet OH$) and ROS (H_2O_2 , $ONOO^-$) are generated endogenously (mitochondria, NOX, XO) and exogenously (smoking, UV, pollution)
- Four major damage targets: Lipid peroxidation (chain reaction; F2-isoprostanes, oxLDL), protein oxidation (carbonylation, 3-NT), DNA oxidation (8-OHdG, strand breaks), glycoxidation (AGEs via RAGE)
- Enzymatic defense ($SOD \rightarrow H_2O_2 \rightarrow$ catalase/GPx) + GSH system + Nrf2/Keap1 transcriptional regulation form the primary defense network
- Oxidative stress drives CVD (endothelial dysfunction, LDL oxidation), cancer (mutagenic DNA lesions, NF- κ B promotion), and T2DM (polyol pathway, β -cell damage, insulin resistance)
- Supplement RCTs largely negative or harmful; whole dietary patterns (Mediterranean, DASH) consistently protective — food matrix and antioxidant network synergy are essential
- Biomarker hierarchy: F2-isoprostanes > 8-OHdG > MDA/TBARS for in vivo oxidative stress assessment

Suggested Reading:

1. Halliwell B & Gutteridge JMC (2015). Free Radicals in Biology and Medicine, 5th Ed. |
2. Sies H et al. (2017) Oxidative stress. Ann Rev Biochem. |
3. Bouayed J & Bohn T (2010) Exogenous antioxidants — double-edged swords. Oxid Med Cell Longev. |
4. Miller ER et al. (2005) Meta-analysis: High-dose vitamin E supplementation may increase all-cause mortality. Ann Intern Med.

LECTURE 2

Phytochemicals: Food Sources & Medicinal Benefits

Classification, Bioavailability, Mechanisms of Action & Chronic Disease Evidence

Nutrition & Dietetics MS Program | Department of Nutritional Sciences

Learning Outcomes — By the end of this lecture, students will be able to:

- Classify phytochemicals into major chemical families and identify key representatives with their food sources
- Describe the mechanisms of action of phytochemicals at the molecular level (Nrf2, NF- κ B, enzyme modulation, receptor interactions)
- Evaluate the epidemiological and clinical trial evidence for phytochemicals in CVD, cancer, and T2DM prevention
- Explain bioavailability-limiting factors and strategies to optimize phytochemical absorption from foods
- Formulate evidence-based dietary counseling recommendations integrating phytochemical-rich food sources

I. INTRODUCTION — PHYTOCHEMICALS IN HUMAN HEALTH

Phytochemicals (phytonutrients) are biologically active non-nutrient compounds in plant foods — estimated >25,000 distinct structures. They are not essential in the classical sense (no deficiency disease defined in humans) but mounting evidence demonstrates their role in chronic disease prevention through multiple overlapping mechanisms. The term 'protective elements' has been adopted in functional nutrition to reflect this role.

Unlike vitamins and minerals, phytochemicals are not uniformly distributed — different plant families, parts of plants, growing conditions, and preparation methods produce dramatically different phytochemical profiles. This underpins the principle of dietary diversity as the optimal phytochemical delivery strategy.

Major Class	Key Subclasses	Representative Compounds
Polyphenols	Flavonoids, phenolic acids, stilbenes, lignans, tannins	Quercetin, EGCG, resveratrol, chlorogenic acid, curcumin
Carotenoids	Xanthophylls, carotenes	Lycopene, lutein, zeaxanthin, astaxanthin, β -cryptoxanthin
Glucosinolates / ITCs	Aliphatic, indole, aromatic glucosinolates	Sulforaphane, I3C/DIM, PEITC, iberin
Organosulfur compounds	Thiosulfinates, thiosulfonates, sulfides	Allicin, DADS, DATS, S-allylcysteine (SAC)
Phytosterols	4-desmethylsterols, methylsterols, triterpenes	Beta-sitosterol, campesterol, stigmasterol

Terpenoids/Terpenes	Monoterpenes, diterpenes, triterpenes	Limonene, ursolic acid, oleanolic acid, carnosol
Alkaloids	Xanthines, indole alkaloids	Caffeine, theobromine, berberine, piperine
Saponins	Steroidal, triterpenoid	Ginsenosides, solanine, avenococides

II. POLYPHENOLS — THE LARGEST & MOST STUDIED CLASS

A. Flavonoids (~6,000 compounds)

Flavonoids share a C6-C3-C6 diphenylpropane skeleton (two benzene rings connected by a 3-carbon chain forming a pyran ring). Subclass variation arises from the degree of oxidation and hydroxylation of the central ring.

1. Flavonols — Quercetin, Kaempferol, Myricetin

Primary Sources: Onions (quercetin, highest in outer rings), kale, broccoli, leeks, apples (skin), capers, berries, tea, red wine

Mechanisms: Nrf2 activator; NF- κ B inhibitor (blocks IKK β phosphorylation); inhibits PI3K/Akt and MAPK; COMT inhibitor (raises catechol estrogen catabolism); inhibits CYP1A1/1B1 (carcinogen activation); chelates Fe²⁺ and Cu⁺; inhibits xanthine oxidase (anti-gout)

Evidence: *Meta-analysis (>30 RCTs): Quercetin supplementation significantly reduces systolic (-3.1 mmHg) and diastolic (-2.2 mmHg) blood pressure and reduces CRP. Higher dietary quercetin intake inversely associated with all-cause mortality and CVD risk in large cohort studies (Nurses' Health Study, EPIC).*

2. Flavanols / Flavan-3-ols — Catechins & Proanthocyanidins

Primary Sources: Green tea (EGCG most abundant flavanol; 50–300 mg/cup), dark chocolate/cocoa (epicatechin), red wine (procyanidins), apples, grapes, berries

Key Compounds: Epicatechin (EC), Epigallocatechin gallate (EGCG — most potent), Epigallocatechin (EGC), Epicatechin gallate (ECG); proanthocyanidins (PACs) = oligomers

EGCG Mechanisms: Inhibits DHFR (dihydrofolate reductase) and DNA methyltransferases (DNMT) → epigenetic reactivation of silenced tumor suppressor genes; inhibits 26S proteasome → pro-apoptotic; activates AMPK; inhibits HER2/ErbB2; inhibits fatty acid synthase (FASN) → anti-lipogenic in cancer cells

Evidence: *Cocoa flavanol RCTs: COSMOS-Mind trial — cocoa flavanol supplementation (600 mg/day cocoa extract) improved memory and global cognition in older adults over 3 years; COSMOS cardiovascular trial — 21% ↓ CVD mortality. Green tea meta-analyses: ~28% ↓ type 2 diabetes incidence; modest ↓ LDL-C (~5 mg/dL).*

Counseling Tip: *Dark chocolate must contain ≥70% cocoa to be clinically meaningful. Milk chocolate binds cocoa flavanols to casein, significantly reducing bioavailability. Green tea is best prepared at 80°C (not boiling) to preserve EGCG; catechin degradation accelerates above 90°C.*

3. Anthocyanins — Berry Pigments

Primary Sources: Blueberries, blackberries, raspberries, strawberries, cherries, black elderberries, red/purple grapes, red cabbage, purple corn, black beans

Mechanisms: Inhibit NF- κ B and COX-2; activate AMPK; cross blood-brain barrier (neuroprotective); modulate gut microbiome ($\uparrow$ Bifidobacterium, $\uparrow$ Akkermansia muciniphila); improve endothelial NO production; protect retinal photoreceptors

Evidence: *Blueberry RCTs: Regular consumption (1 cup/day, 6 weeks) significantly improves FMD (flow-mediated dilation) — marker of endothelial function — in adults with MetS. Anthocyanin intake inversely associated with T2DM risk (RR 0.85, 95% CI) in large prospective cohorts. Elderberry extract reduces cold/flu duration by ~2 days (meta-analysis, Cochrane-level evidence).*

4. Flavanones — Citrus Bio actives

Primary Sources: Oranges and orange juice (hesperidin), grapefruit (naringenin, naringin), lemons (eriocitrin); found predominantly in inner peel (albedo) and membranes — lost when juicing

Mechanisms: Naringenin: β -oxidation upregulation (PPAR α agonist-like); inhibits de novo lipogenesis; CYP3A4 inhibition by naringenin (grapefruit interaction — significant drug interactions with statins, immunosuppressants, CCBs)

Clinical Note: *Grapefruit-drug interaction: Furanocoumarins (bergamottin, DHB) in grapefruit juice irreversibly inhibit intestinal CYP3A4 $\rightarrow$ markedly $\uparrow$ bioavailability of CYP3A4 substrates (simvastatin, cyclosporine, amlodipine, midazolam). Interaction can persist 24-72 hours. Clinically significant counseling point.*

5. Isoflavones — Soy Phytoestrogens

Primary Sources: Soybeans, edamame, tofu, tempeh (highest bioavailability), miso, soy milk; red clover, kudzu

Compounds: Genistein (most studied), daidzein, glycitein; inactive glucosides in food $\rightarrow$ hydrolyzed by gut β -glucosidase to aglycones; daidzein metabolized by gut bacteria to equol (more potent; only ~30% Western adults are equol producers)

Mechanisms: Selective Estrogen Receptor Modulator (SERM)-like activity: bind ER α and ER β (preferentially ER β) with lower affinity than estradiol; agonist/antagonist depending on tissue estrogen milieu; inhibit tyrosine kinases (EGFR, HER2); inhibit aromatase; induce G2/M cell cycle arrest; induce BRCA1/2 expression

Evidence: *Soy consumption (Asian cohorts, 1-2 servings/day): Inversely associated with breast cancer (ER-negative subtypes) and prostate cancer incidence. Western supplemental isoflavone data more mixed. Menopausal symptoms: modest reduction in hot flash frequency (~25%) in RCTs. WHO and major dietetics bodies consider moderate soy food consumption safe for breast cancer survivors.*

6. Stilbenes — Resveratrol

Primary Sources: Red grape skin (0.1–1.8 mg/glass red wine), peanuts, mulberries, Japanese knotweed (highest concentration; supplement source)

Mechanisms: SIRT1 direct activation (and indirect via AMPK $\rightarrow$ $\uparrow$ NAD $^{+}$ /NADH ratio); PGC-1 α deacetylation $\rightarrow$ mitochondrial biogenesis; inhibits NF- κ B (IKK β); inhibits PI3K, mTORC1; inhibits CYP1A1/1B1; inhibits platelet TXA $_2$ synthesis; HDAC inhibitor; telomere protection via TRF2

Evidence: *Clinical translation remains limited due to poor oral bioavailability ($t_{1/2} < 30$ min; rapid sulfation/glucuronidation). High-dose supplements (1-2 g/day) achieve micromolar plasma levels but tissue concentrations unknown. Some RCTs show benefit in T2DM and NAFLD; cardiovascular outcome trials largely neutral. Resveratrol in red wine insufficient to explain French Paradox (<2 mg/glass).*

B. Phenolic Acids

Primary Sources: Coffee (chlorogenic acids — single largest polyphenol source in Western diets, >1g/day in regular coffee drinkers), berries, whole grains, artichokes, potatoes

Mechanisms: Hydroxycinnamic acids (caffeic, ferulic, chlorogenic): Nrf2 activation; inhibit α -glucosidase and α -amylase → ↓ postprandial glucose; improve insulin sensitivity (AMPK); gut microbiome modulation

Evidence: *Coffee consumption (3-4 cups/day) inversely associated with T2DM risk (~25-30% ↓), Parkinson's disease, Alzheimer's, liver cirrhosis, and hepatocellular carcinoma. Both caffeinated and decaffeinated coffee protective — implicating chlorogenic acids and diterpene antioxidants rather than caffeine alone. EPIC cohort: ~15% ↓ all-cause mortality in moderate coffee drinkers.*

C. Lignans

Primary Sources: Flaxseeds (richest source, ~300 mg SDG/100g), sesame seeds, whole grains, legumes, cruciferous vegetables

Metabolism: Secoisolariciresinol diglucoside (SDG) in flaxseed → gut bacterial metabolism (*Clostridium scindens*, Lactonifactor) → enterodiol and enterolactone (mammalian lignans with weak estrogenic activity)

Evidence: *Higher enterolactone plasma levels inversely associated with breast cancer risk and cardiovascular mortality in prospective cohort studies. Lignan intake may improve lipid profiles and reduce breast cancer recurrence in postmenopausal women (early RCT data). Flaxseed (1 tbsp/day ground) also provides ALA (ω -3), fiber, and lignans — synergistic benefit for CVD and T2DM.*

III. CAROTENOIDS — BEYOND PROVITAMIN A

Carotenoid	Top Food Sources	Key Mechanisms & Evidence
Lycopene	Cooked tomatoes, tomato paste, watermelon, pink grapefruit, guava	Potent $^1\text{O}_2$ quencher; inhibits IGF-1 signaling; Nrf2 activator; gap junction upregulation (anti-proliferative). Epidemiology: $\geq 40\%$ $\downarrow$ prostate cancer risk in high lycopene consumers. Absorption $\uparrow\uparrow$ with fat + heating (cis-isomers more bioavailable from cooked tomatoes)
Lutein + Zeaxanthin	Kale, spinach, collards, corn, egg yolk, orange peppers	Macular pigment optical density (MPOD); blue light filter; quench $^1\text{O}_2$ in retina. RCT: AREDS2 $\downarrow$ 25% AMD progression. Emerging cognitive data: COSMOS-Mind, Carotenoids & Cognition studies show brain lutein (DHA-bound) correlates with processing speed and memory in older adults
Astaxanthin	Wild salmon, krill, shrimp, lobster, microalgae Haematococcus pluvialis	10-100x more potent antioxidant than β -carotene; crosses BBB and blood-retinal barrier; inhibits 5-LOX and COX-2; anti-fatigue in athletes (RCTs); neuroprotective; improving NAFLD in early trials
β -Cryptoxanthin	Mandarins/tangerines, oranges, papayas, paprika, pumpkin	Provitamin A activity; anti-inflammatory; inverse association with lung cancer, rheumatoid arthritis, and bone loss in prospective cohorts

IV. GLUCOSINOLATES, ORGANOSULFUR & TERPENOIDS

A. Glucosinolates & Isothiocyanates (Cruciferous Vegetables)

Glucosinolates are S-containing glycosides hydrolyzed by myrosinase (plant enzyme released on cell damage — chopping, chewing) or by colonic microbiota to isothiocyanates (ITCs), indoles, and nitriles.

Compound / Source	Mechanism & Clinical Evidence
Sulforaphane (SFN) — broccoli, broccoli sprouts (10-100x richer than mature heads)	Most potent dietary Nrf2 activator: thiocarbamate-mediated Keap1 Cys151 modification → ARE-driven Phase II enzyme induction (GST, NQO1, HO-1, GCL, GCLM). Anti-cancer (Phase II trials in prostate, breast, bladder). Anti-inflammatory: inhibits NF-κB, HDAC inhibitor (↑ tumor suppressor gene expression). Phase 2/3 trial data emerging in autism spectrum disorder (GI symptoms, behavior). Anti-Helicobacter pylori activity.
DIM (diindolylmethane) from I3C — all cruciferous vegetables	Aryl hydrocarbon receptor (AhR) ligand; modulates estrogen metabolism (↑ 2-OHE vs 16α-OHE ratio); inhibits CDK2/4 → G1 cell cycle arrest; anti-angiogenic (VEGF inhibition). Early clinical trials in cervical dysplasia and breast cancer prevention.
PEITC — watercress, garden cress	Inhibits CYP2E1 and CYP2A6 (tobacco carcinogen activation); Phase 2 human trial: single watercress meal significantly reduced urinary NNK metabolites in smokers. Induces apoptosis in lung cancer cells via ROS-dependent mechanism.
Sinigrin → allyl isothiocyanate (AITC) — mustard, horseradish, wasabi	TRPA1 channel agonist (pungency); antimicrobial; inhibits cancer cell migration and invasion; anti-inflammatory via NF-κB.

Counseling Tip: Maximize sulforaphane from broccoli: (1) Chop/chew raw or lightly steam (don't boil — destroys myrosinase); (2) Add raw mustard seed powder or daikon radish to cooked broccoli (provides exogenous myrosinase); (3) Broccoli sprout consumption is most potent single dietary source — 30-50g sprouts per day used in clinical trials.

B. Organosulfur Compounds — Allium Vegetables

All allium vegetables (garlic, onions, leeks, shallots, chives, scallions) contain S-alk(en)yl-cysteine sulfoxides. Alliinase (activated by cell damage) converts alliin → allicin (unstable; rapidly converts to diallyl disulfide/DADS, diallyl trisulfide/DATS, ajoene, vinylthiins in vivo).

Compound & Best Source	Key Actions
Allicin (fresh crushed garlic)	Thiol-reactive; inhibits thiol-dependent enzymes in bacteria and fungi (antimicrobial broad-spectrum, including MRSA, H. pylori); anti-thrombotic (inhibits platelet TXA ₂); inhibits HMG-CoA reductase (statin-like); unstable — destroyed by heat, acid
DATS (diallyl trisulfide) — garlic oil	Potent apoptosis inducer in cancer cells via mitochondrial pathway (activates intrinsic caspases); inhibits HDAC; crosses BBB; neuroprotective in preclinical models
S-Allylcysteine (SAC) — aged garlic extract	Water-soluble, stable, highest bioavailability of all garlic sulfur compounds; AMPK activator; antioxidant (Nrf2); cardiovascular

	RCTs: reduces central blood pressure, arterial stiffness, LDL oxidation; immune modulation
Quercetin in onions (synergy)	Onion's flavonol quercetin + organosulfur compounds act synergistically on cardiovascular biomarkers and NF-κB pathway — food matrix synergy example

Evidence: *Garlic supplementation meta-analysis (39 RCTs): Significantly reduces total cholesterol (-17 mg/dL), LDL-C (-9 mg/dL), triglycerides (-11 mg/dL), and systolic BP (-3.75 mmHg). Aged garlic extract (AGE) shows most consistent evidence — standardized, bioavailable, odor-free. Kyolic brand AGE used in many RCTs.*

C. Phytosterols — Cholesterol Competitors

Primary Sources: Plant oils (especially wheat germ, sesame, corn oils), nuts (especially pistachios, almonds), seeds, whole grains, legumes; fortified foods (margarines, orange juice, yogurt)

Mechanisms: Structural analogs of cholesterol; compete for intestinal micelle incorporation and NPC1L1 transporter uptake → reduce intestinal cholesterol absorption by ~30-50%; upregulate hepatic LDL receptor expression (secondary to reduced sterol delivery)

Evidence: *Plant sterol esters 2g/day → ~10% ↓ LDL-C (meta-analysis, >100 RCTs). FDA-approved health claim. European Society of Cardiology includes phytosterols as first-line lifestyle intervention for hypercholesterolemia. Particularly effective when combined with Mediterranean diet. Note: reduce absorption of fat-soluble vitamins and carotenoids at high doses — advise adequate carotenoid-rich food intake.*

D. Curcuminoids — Turmeric

Primary Sources: Turmeric root (*Curcuma longa*) — 2-5% curcuminoids by dry weight; curry powder (variable); black pepper piperine as bioenhancer (BioPerine®: inhibits CYP3A4 + UGT → ↑ curcumin t_{1/2} by 20-fold)

Mechanisms: NF-κB inhibitor (binds IKKβ); Nrf2 activator; HDAC inhibitor; mTOR inhibitor; AMPK activator; inhibits COX-2 and 5-LOX; inhibits EGFR; induces apoptosis (Bax/Bcl-2 ratio shift); anti-amyloid (disaggregates Aβ and tau fibrils in vitro); PPARγ agonist (insulin sensitizing)

Evidence: *Major limitation: Extremely low bioavailability (rapid conjugation, poor solubility). >120 completed clinical trials. Positive results in: NAFLD/NASH (improved ALT, steatosis), knee OA (comparable to NSAIDs, 1g/day, 8 weeks), T2DM prevention (pre-diabetes RCT: ↓ T2DM conversion by 78% vs placebo over 9 months, n=240). Nanoparticle, liposomal, and phospholipid formulations under investigation for improved delivery.*

Counseling Tip: *Standard turmeric in cooking delivers minimal pharmacological levels. For therapeutic benefit, bioavailability-enhanced curcumin supplements (piperine-containing, lipid-formulated, or nanoparticles) are needed. Counsel patients on standardized products with defined curcuminoid content.*

E. Other Notable Phytochemicals

Berberine

Alkaloid from Berberis (barberry), goldenseal, Oregon grape, Coptis. Activates AMPK (comparable to metformin mechanistically — inhibits mitochondrial Complex I → ↑ AMP:ATP → AMPK). Inhibits PCSK9 (increases LDL receptor recycling). Gut microbiome modulator (↑ short-chain fatty acid producers). RCT evidence: reduces fasting glucose, HbA1c, triglycerides, and LDL-C — comparable to metformin in some head-to-head T2DM trials. Growing evidence in PCOS, NAFLD.

Quercetin & Fisetin — Senolytics

Emerging evidence that quercetin (with dasatinib) and fisetin (in strawberries, apples, persimmons) selectively induce apoptosis in senescent cells — cells that accumulate with aging and drive chronic inflammation ('inflammaging'). Early-phase clinical trials in aging-related conditions (chronic kidney disease, frailty, COVID long-haul). Fisetin is particularly BBB-penetrant; promising in neurodegeneration models.

Piperine

From black pepper (*Piper nigrum*). TRPV1 agonist (thermogenic, enhances absorption). Most importantly: potent CYP3A4 and P-glycoprotein (P-gp) inhibitor → acts as universal bioenhancer for many phytochemicals (curcumin, resveratrol, CoQ10). Clinically relevant: can alter drug pharmacokinetics — advise caution with narrow therapeutic index drugs.

V. BIOAVAILABILITY — THE CRITICAL DETERMINANT

A. Factors Affecting Phytochemical Bioavailability

Factor	Effect & Clinical Relevance
Food matrix	Cell wall entrapment reduces release; cooking/processing disrupts cell walls → ↑ bioavailability for most carotenoids and lycopene; BUT destroys myrosinase for glucosinolates
Dietary fat co-ingestion	Fat-soluble phytochemicals (carotenoids, fat-soluble polyphenols, curcumin): absorption 2-10x higher when eaten with 3-5g fat; low-fat salad dressings with salads significantly reduce carotenoid absorption
Gut microbiome	Major determinant for many polyphenols: only ~30% of Western adults produce equol from isoflavones; urolithin production from ellagitannins varies 0-100% by individual microbiome; enterolactone from lignans depends on <i>Clostridium scindens</i> abundance
Phase II metabolism	Rapid glucuronidation, sulfation, methylation in intestinal epithelium and liver limits plasma levels; aglycone forms (deglycosylated) generally more bioavailable than glycosides in food
Processing & cooking	Boiling leaches water-soluble polyphenols; fermentation (↑ bioavailability via deglycosylation, ↓ phytate); soaking legumes reduces phytate 30-70%
Gastric pH	Achlorhydria or PPI use alters polyphenol stability and microbial fermentation pattern in proximal gut
Timing & frequency	Frequent small doses maintain more sustained plasma levels than single large dose; antioxidant capacity peaks 1-3 hours post-consumption

B. Gut Microbiome — The Phytochemical Transformer

The gut microbiome dramatically transforms dietary phytochemicals, often converting inactive or poorly bioavailable forms to potent bioactive metabolites. This creates substantial inter-individual variation in phytochemical response:

- Ellagitannins (pomegranate, walnuts, raspberries) → urolithins (A, B, C) by *Gordonibacter pamelaeae* and *Ellagibacter isourolithinifaciens*; Urolithin A activates mitophagy and mitochondrial biogenesis (PINK1/Parkin pathway); clinical trials showing muscle performance improvement in older adults
- Isoflavones (daidzein) → equol by *Slackia isoflavoniconvertens* and *Lactococcus* sp.; equol is more potent phytoestrogen; produced by ~30% Western adults, ~50-60% Asian adults; explains population differences in soy health effects
- Lignans (SDG from flaxseed) → enterodiol → enterolactone by *Lactonifactor longoviformis* and *Blautia producta*; enterolactone inversely associated with breast cancer and CVD risk
- Resveratrol → dihydroresveratrol and lunularin by colonic bacteria; these metabolites may be partly responsible for biological effects attributed to resveratrol
- Quercetin glycosides → quercetin aglycone (deglycosylation by β -glucosidase of *Lactobacillus*, *Bacteroides*); then ring-fission to protocatechuic acid, phloroglucinol

Counseling Tip: *Probiotic-rich fermented foods (yogurt, kefir, kimchi, sauerkraut, tempeh) may enhance phytochemical bioavailability by improving the microbiome's transformative capacity. Advise simultaneous dietary fiber (prebiotic) to support equol- and urolithin-producing bacteria.*

VI. CHRONIC DISEASE PREVENTION — CLINICAL EVIDENCE SYNTHESIS

A. Cardiovascular Disease

Phytochemical	Key CVD Evidence
Polyphenols (overall)	PREDIMED trial (n=7447): Mediterranean diet + olive oil (↑ polyphenols) → 30% ↓ major CVD events. Polyphenol intake inversely associated with CVD mortality in EPIC cohort (HR 0.82 per SD increase)
Flavanols (cocoa)	COSMOS trial: Cocoa extract 600mg/day → 21% ↓ CVD mortality over 3.6 years. Endothelial function (FMD) improved in 22 RCTs (WMD +1.34%).
Olive oil polyphenols (oleocanthal, hydroxytyrosol)	Hydroxytyrosol reduces oxLDL, platelet aggregation; oleocanthal inhibits COX-1/2 (ibuprofen-like); extra-virgin olive oil (EVOO) ≥ Phenol content ≥ 200 mg/kg linked to greater CVD protection vs low-phenol olive oil
Plant sterols	2g/day → 10% ↓ LDL-C; FDA-approved health claim; ESC Class IIa recommendation
Garlic (AGE)	39-RCT meta-analysis: ↓ TC (-17mg/dL), LDL (-9mg/dL), TG (-11mg/dL), SBP (-3.75mmHg)
Omega-3 (EPA from marine foods)	REDUCE-IT: Icosapentaenoic acid 4g/day → HR 0.75 for major CVD events beyond statin therapy; ~45% ↓ TG

B. Cancer Prevention

Phytochemical	Key Cancer Evidence
Sulforaphane / Cruciferous	Epidemiological: 2-3 servings cruciferous/week → ~30% ↓ bladder, lung, colorectal cancer. Mechanistic RCTs: SFN (200μmol/day) reduced PSA velocity in men with recurrent prostate cancer; broccoli sprout extract reduced aflatoxin-DNA adducts in Chinese cohort (field chemopreventive trial)
Lycopene	Prospective cohort: Highest lycopene quartile → ~40% ↓ prostate cancer; particularly for aggressive disease. 12-study meta-analysis confirms inverse association (RR 0.81). Prostate Lycopene RCT (n=40): 15mg/day → reduced PSA, Ki67 proliferation index, oxidative DNA damage pre-surgically
EGCG (green tea)	Asian cohort studies: 5+ cups/day green tea → ~30% ↓ colorectal cancer, significant ↓ breast cancer recurrence in early-stage patients. Phase 2 trial (CLL): EGCG 400-2000mg/day → 50% reduction in absolute lymphocyte count in 33% of patients
Isoflavones	Shanghai Women's Health Study: Soy intake inversely associated with breast cancer (non-significant trend). Greater effect in pre-menopausal and ER-negative subtypes. WCRF recommends moderate soy food intake as safe for breast cancer survivors
Curcumin	Phase 2: Bioavailability-enhanced curcumin in colorectal cancer patients: detectable in colonocytes; reduced ACF (aberrant crypt foci). Phase 3 trials ongoing in colon, pancreatic cancer

C. Type 2 Diabetes & Metabolic Syndrome

Phytochemical	Mechanism & Evidence
Flavonoids (quercetin, anthocyanins, EGCG)	Inhibit α -glucosidase and α -amylase (reduce glucose absorption); activate AMPK ($\uparrow$ glucose uptake); improve insulin receptor signaling; reduce adipokine imbalance. Dietary flavonoid intake inversely associated with T2DM risk in Nurses' Health Study (RR 0.74, highest vs lowest quintile)
Chlorogenic acid (coffee)	Inhibits hepatic glucose-6-phosphatase $\rightarrow$ $\downarrow$ hepatic glucose output; $\downarrow$ glucose transport; activates AMPK. Meta-analysis (28 cohort studies): 3-4 cups coffee/day $\rightarrow$ 25% $\downarrow$ T2DM risk
Curcumin	PPAR γ agonist; AMPK activator; $\downarrow$ adipogenesis; $\downarrow$ TNF- α -induced insulin resistance; improves HOMA-IR. Pre-diabetes RCT: 78% $\downarrow$ T2DM conversion vs placebo over 9 months
Berberine	AMPK activation (metformin-like); PCSK9 inhibition; gut microbiome modulation. Meta-analysis (14 RCTs): Berberine comparable to metformin for FPG, HbA1c, and TG reduction in T2DM patients
Resveratrol	SIRT1 $\rightarrow$ PGC-1 α deacetylation $\rightarrow$ mitochondrial function; improves insulin sensitivity in T2DM RCTs (mixed results); meta-analysis: significant $\downarrow$ FPG, HOMA-IR, HbA1c in T2DM subgroup

Lecture 2 Summary — Key Principles

- Phytochemicals encompass >25,000 compounds across polyphenols, carotenoids, glucosinolates, organosulfur compounds, sterols, and alkaloids — each with distinct food sources and mechanisms
- Core mechanisms: Nrf2/ARE activation (Phase II enzymes), NF- κ B inhibition (anti-inflammatory), enzyme modulation (CYP inhibition, α -glucosidase, HMG-CoA), AMPK activation, SIRT1 activation, epigenetic modification (HDAC inhibition, DNA methylation)
- Bioavailability is the critical determinant — gut microbiome transforms phytochemicals to bioactive metabolites (urolithins, equol, enterolactone); inter-individual variation is substantial
- CVD evidence strongest for Mediterranean dietary pattern (PREDIMED), cocoa flavanols (COSMOS), plant sterols, and garlic
- Cancer chemopreventive evidence strongest for sulforaphane (Nrf2/Phase II), lycopene (prostate), and cruciferous vegetables (epidemiology + mechanistic data)
- T2DM evidence: dietary flavonoids, chlorogenic acid (coffee), curcumin (pre-diabetes), and berberine (metformin-comparable in RCTs) show consistent benefit
- Whole food sources outperform isolated supplements due to bioavailability, food matrix effects, and microbiome-mediated activation

Suggested Reading:

1. Manach C et al. (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr. |
2. Dinkova-Kostova AT & Talalay P (2008) Direct and indirect antioxidant properties of inducers of cytoprotective proteins. Mol Nutr Food Res. |
3. Rauf A et al. (2018) Berberine pharmacological activities. Biomed Pharmacother. |
4. Lampe JW (2003) Isoflavonoid and lignan phytoestrogens as dietary biomarkers. J Nutr.

LECTURE 3

Fruits & Vegetables: Essential Micronutrient Sources

Nutritional Composition, Bioavailability, Public Health Evidence & Counseling Strategies

Nutrition & Dietetics MS Program | Department of Nutritional Sciences

Learning Outcomes — By the end of this lecture, students will be able to:

- Profile the micronutrient and phytochemical composition of major fruit and vegetable groups
- Explain how processing, cooking, and storage affect micronutrient retention in fruits and vegetables
- Describe the epidemiological evidence linking fruit and vegetable consumption to CVD, cancer, and T2DM risk reduction
- Identify specific fruits and vegetables with particularly high clinical relevance for micronutrient delivery
- Design evidence-based, practical dietary counseling strategies to increase fruit and vegetable intake across diverse populations

I. THE CASE FOR FRUITS & VEGETABLES — PUBLIC HEALTH CONTEXT

A. Global Consumption Gap & Disease Burden

Fruits and vegetables (F&V) are arguably the most nutrient-dense food groups per calorie — providing vitamins, minerals, dietary fiber, and thousands of phytonutrients with minimal energy density. Despite this, global F&V consumption remains critically below recommendations:

- Global: WHO recommends $\geq 400\text{g}$ F&V/day (excluding starchy vegetables); $>50\%$ of the global population fails to meet this target
- United States: Only $\sim 9\text{-}12\%$ of American adults consume recommended F&V servings (CDC 2022); average daily intake ~ 1.5 cups F&V combined
- Economic inequity: F&V consumption strongly correlated with income, education, and food access — healthy F&V are 3-6x more expensive per calorie than ultra-processed alternatives in many food systems
- Disease burden attributable to low F&V: WHO estimates ~ 5.2 million deaths/year attributable to insufficient fruit and ~ 2.7 million to insufficient vegetable consumption — 4th leading dietary risk factor globally

Evidence: *Global Burden of Disease Study (Aune et al., Lancet 2016, $n > 2.5$ million): Every additional 80g serving of F&V/day associated with 8% ↓ CVD mortality, 6% ↓ cancer mortality, 7% ↓ all-cause mortality. Optimal benefit at 5-7 servings/day with little additional gain beyond 7-8.*

B. Recommended Intakes — Key Guidelines

Guideline / Authority	Fruit Recommendation	Vegetable Recommendation
WHO/FAO	250g/day min	150g/day min (5+ servings total)
US Dietary Guidelines 2020-25	1.5-2 cups/day	2-3 cups/day (half the plate)
AICR (Cancer Prevention)	Implied in plant-food emphasis	2/3 plate plant foods recommended
EAT-Lancet Planetary Health	200-300g/day	300-600g/day (including legumes)
DASH Diet	4-5 servings/day	4-5 servings/day
Mediterranean Diet	2-3 servings/day	6-8 servings/day (vegetables dominant)

II. NUTRITIONAL COMPOSITION — MAJOR F&V GROUPS

A. Dark Leafy Green Vegetables

Among all food groups, dark leafy greens offer the highest micronutrient density per calorie. Key representatives: spinach, kale, Swiss chard, collard greens, bok choy, arugula, watercress, romaine lettuce, beet greens.

Nutrient	Key Sources (per 100g raw)	Clinical Significance
Vitamin K1 (phylloquinone)	Kale: 705µg; spinach: 483µg; chard: 830µg	Coagulation, osteocalcin carboxylation, MGP (vascular calcification prevention); AI = 90-120µg/day
Folate (B9)	Spinach: 194µg DFE; asparagus: 52µg	NTD prevention, DNA synthesis, 1C metabolism; RDA = 400µg DFE; critical in pregnancy
Vitamin C	Kale: 93mg; watercress: 43mg; bok choy: 45mg	Collagen synthesis, iron absorption enhancer, aqueous antioxidant; RDA = 75-90mg/day
Lutein + Zeaxanthin	Kale: 39.5mg; spinach: 12.2mg; collards: 14.6mg	Macular pigment; AMD and cataract prevention; no DRI established; ≥10mg/day lutein studied in AREDS2
Magnesium	Spinach: 79mg; Swiss chard: 81mg	ATP synthesis, >300 enzyme cofactor; RDA = 310-420mg/day; commonly deficient
Iron (non-heme)	Spinach: 2.7mg; chard: 1.8mg	Must be consumed with vitamin C to optimize non-heme absorption; RDA = 8-18mg/day

Calcium	Bok choy: 105mg (high bioavailability); kale: 150mg	Better absorbed than spinach calcium (low oxalate); bok choy absorption ~50% vs dairy ~32%
Beta-carotene (β-carotene)	Kale: 9.2mg; spinach: 5.6mg; Swiss chard: 3.6mg	Provitamin A (RAE conversion); antioxidant; absorption ↑ with fat
Vitamin E (α-tocopherol)	Spinach: 2.0mg; Swiss chard: 1.9mg	Membrane antioxidant; RDA = 15mg/day; needs fat for absorption

Clinical Note: *Spinach calcium paradox: Spinach is high in calcium (99mg/100g) but also very high in oxalate, which precipitates calcium in the gut — absorption rate is only ~5-8%. Contrast with bok choy (lower calcium but ~50% absorption rate due to very low oxalate). Critical for counseling vegetarians/vegans about calcium sources.*

B. Orange & Yellow Vegetables

Richest sources of provitamin A carotenoids (β-carotene, α-carotene) and vitamin C. Key representatives: carrots, sweet potato, butternut squash, pumpkin, orange/yellow bell peppers, corn, yellow tomatoes.

Food (100g cooked)	Key Micronutrients	Special Notes
Sweet potato	Beta-carotene: 9.4mg; Vitamin C: 20mg; Potassium: 475mg; Manganese: 0.5mg	Higher glycemic index than regular potato but higher micronutrient density; skin provides additional fiber and phenolics; one medium sweet potato ≈ 100% daily vitamin A
Carrots	Beta-carotene: 8.3mg; Vitamin K: 13μg; Potassium: 320mg	Cooking ↑ β-carotene bioavailability by breaking cell walls; fat co-ingestion essential for carotenoid absorption; purple carrots contain anthocyanins (additional benefit)
Butternut squash	Beta-carotene: 4.6mg; Vitamin C: 21mg; Vitamin B6: 0.2mg; Folate: 27μg	Excellent all-around micronutrient profile; low calorie density (45 kcal/100g); manganese cofactor for MnSOD
Yellow/orange bell pepper	Vitamin C: 184mg (highest of all peppers); Vitamin B6: 0.3mg; Vitamin E: 1.6mg	Far exceeds orange in vitamin C content; capsanthin (carotenoid); excellent raw snack for vitamin C delivery
Pumpkin	Beta-carotene: 3.1mg; Potassium: 230mg; Zinc: 0.3mg	Pumpkin seeds rich in Zn, Mg, phytosterols; cucurbitacins in flesh (anti-cancer in preclinical studies)

C. Cruciferous Vegetables

Beyond their phytochemical profile (glucosinolates — Lecture 2), cruciferous vegetables are exceptional micronutrient sources. Family: broccoli, Brussels sprouts, cauliflower, cabbage, kale, bok choy, arugula, radish, turnips.

Nutrient & Top Cruciferous Sources	Amount & Clinical Note
Vitamin C — Broccoli (89mg/100g raw), Brussels sprouts (85mg), Cauliflower (48mg)	Exceeds orange; largely preserved by steaming; RDA = 75-90mg/day met by ½ cup raw broccoli
Vitamin K1 — Kale (705µg), Brussels sprouts (177µg), Broccoli (102µg)	Major dietary K1 source; consistency of intake important for patients on warfarin
Folate — Broccoli (63µg/100g), Brussels sprouts (61µg)	Significant contributor to folate intake; preferably raw or lightly cooked (folate is heat-labile)
Calcium — Bok choy (105mg; high bioavailability), Kale (150mg; ~50% absorption)	Critical calcium source for vegans; bioavailability superior to spinach (low oxalate)
Potassium — Brussels sprouts (389mg), Broccoli (316mg)	DASH diet mineral; blood pressure regulation; counteracts Na-induced hypertension
Glucosinolates / Sulforaphane — Broccoli sprouts (1000mg/100g), Broccoli (65mg)	Nrf2 activation, Phase II enzymes, cancer chemopreventive; maximize by raw/light steam

D. Allium Vegetables

Garlic, onions, leeks, shallots, chives, scallions. Rich in organosulfur compounds (Lecture 2), quercetin, fructooligosaccharides (FOS — prebiotic), chromium, and selenium (soil-dependent).

- Garlic: Allicin precursors (alliin); selenium (varies by soil); vitamin B6 (1.23mg/100g — 70% RDA); manganese (1.67mg — 87% RDA); vitamin C (31mg); FOS (prebiotic → Bifidobacterium, Lactobacillus ↑)
- Onions: Quercetin (highest in outer layers and red/yellow varieties); fructans (prebiotic fiber); chromium (glucose tolerance factor); folate; potassium
- Leeks: Folate (64µg/100g); vitamin K (47µg); manganese; polyphenols; kaempferol (flavonol with anti-cancer properties in epidemiological studies)

Counseling Tip: Raw garlic maximizes allicin generation (myrosinase analogy: alliinase activated by cell damage; destroyed by heat). Advise: crush/mince garlic and allow 10-minute rest before adding to heat — this allows allicin to form and stabilizes it to brief cooking. Aged garlic extract (AGE) is preferred for supplemental use — standardized, odor-free, bioavailable S-allylcysteine.

E. Berries & Small Fruits

Nutritional powerhouses with exceptional antioxidant capacity. Blueberries, strawberries, raspberries, blackberries, cranberries, elderberries, pomegranate, goji berries, acai.

Berry (100g fresh)	Standout Nutrients	Key Health Associations
Blueberries	Anthocyanins: 163mg; Vitamin C: 10mg; Vitamin K: 19µg; Manganese: 0.3mg	CVD: ↑ FMD, ↓ arterial stiffness (RCTs). Cognition: USDA/Tufts studies; reduces age-related cognitive decline. T2DM: ↓ insulin resistance (bioactive anthocyanins).
Strawberries	Vitamin C: 59mg (79% RDA per 100g); Folate: 24µg; Fisetin (senolytic); Anthocyanins; Ellagic acid	Vitamin C density; fisetin (senolytic activity); ellagic acid → urolithin via gut bacteria. CVD: ↓ LDL-C, ↓ CRP in RCTs.
Raspberries	Fiber: 6.5g; Vitamin C: 26mg; Ellagitannins; Quercetin	Highest fiber content of common berries; ellagitannins → urolithins; prebiotic fiber ↑ Bifidobacterium.
Cranberries	PACs (type-A proanthocyanidins): prevent P-fimbriated E. coli UTI adhesion; Vitamin C: 14mg; Vitamin E: 1.2mg	UTI prevention (Type A PACs — anti-adhesive, not antibiotic); 36mg PACs/day effective in RCTs. Cardiovascular: ↓ LDL oxidation.
Pomegranate	Ellagitannins (punicalagins): 300-400mg; Vitamin C: 10mg; Folate: 38µg; Punicic acid (ω-5 FA)	Urolithin production (gut-dependent); anti-inflammatory (NF-κB); ↓ prostate cancer progression (PSA doubling time extended in Phase 2 trial); ↓ arterial plaque in 3-year RCT.
Elderberries	Anthocyanins: >1500mg/100g (highest); Vitamin C: 36mg; Vitamin A precursors	Immune modulation: NF-κB and cytokine modulation; meta-analysis (elderberry supplementation): ~2 day reduction in cold/flu duration and severity.

F. Citrus Fruits

Oranges, lemons, limes, grapefruit, clementines, tangerines, pomelo. Vitamin C dominates — historically most important for scurvy prevention. Also provide folate, potassium, hesperidin, naringenin, limonoids, and pectin (soluble fiber).

- Vitamin C: One medium orange provides 70mg (≥95% RDA); grapefruit: 78mg; kiwi outperforms citrus (93mg/100g)
- Flavanones: Hesperidin (oranges) and naringenin (grapefruit); anti-inflammatory, lipid-lowering; bioavailability enhanced by consuming whole fruit vs juice (pectin slows transit → ↑ colonic fermentation to bioactive metabolites)
- Folate: Oranges provide 30-40µg/100g; important for periconceptional intake; orange juice fortified with folate is significant contributor in US diet
- Potassium: Grapefruit: 135mg; orange: 181mg; important for DASH diet blood pressure targets
- Limonoids: Limonin, nomilin (in seeds and albedo); induce Phase II enzymes (GST) via Nrf2 in animal models; limited human data but promising anticarcinogenic properties

Clinical Note: Whole fruit vs fruit juice: Juice removes fiber, concentrates sugars, and accelerates gastric emptying → higher glycemic index. The Nurses' Health Study found 3 servings/week of whole fruit (especially blueberries, grapes, apples) reduced T2DM risk by 26%, while fruit juice consumption was ASSOCIATED WITH INCREASED risk. Counsel whole fruit over juice consistently.

G. Tomatoes & Nightshades

Tomatoes, peppers, eggplant, tomatillos. Tomatoes are the dominant dietary lycopene source in Western populations.

- Lycopene: Raw tomato ≈3.0mg/100g; canned crushed tomatoes ≈17mg/100g; tomato paste ≈45mg/100g; cooking breaks down cell walls and converts trans-lycopene → cis-lycopene (more bioavailable); fat co-ingestion essential (incorporate into olive oil-based sauces for maximal lycopene absorption)
- Vitamin C: Bell peppers: 128-184mg/100g (highest among vegetables); red > orange > yellow > green; capsaicin in hot peppers activates TRPV1 → thermogenic, anti-inflammatory, reduces substance P → analgesic applications
- Potassium: Tomatoes 237mg/100g; important for hypertension management
- Vitamin K: 7.9µg/100g (raw tomato)
- Lycopene-prostate evidence: Prospective cohort data (Health Professionals Follow-up Study): Highest vs lowest lycopene intake → RR 0.60 for advanced prostate cancer. Tomato product intake inversely associated across 11 of 14 prospective studies reviewed.

H. Legumes — The Overlooked F&V

Although classified as protein foods in some frameworks, legumes (beans, lentils, chickpeas, peas, soybeans) are exceptionally F&V-like in their micronutrient profile and are featured in all plant-based and Mediterranean dietary patterns as daily staples.

Legume (100g cooked)	Standout Micronutrients	Health Significance
Lentils	Folate: 181µg (45% RDA); Iron: 3.3mg; Zinc: 1.3mg; Potassium: 369mg; Thiamin: 0.17mg	Highest folate density among common foods; combined iron + folate: ideal for IDA + megaloblastic anemia prevention; prebiotic fiber ↑ Bifidobacterium
Black beans	Folate: 149µg; Iron: 2.1mg; Magnesium: 70mg; Anthocyanins in seed coat; Molybdenum	Anthocyanins in black bean coat (rare non-fruit anthocyanin source); molybdenum cofactor for xanthine oxidase, sulfite oxidase
Chickpeas	Folate: 172µg; Zinc: 1.5mg; Manganese: 1.7mg (85% DV); Iron: 2.9mg; Vitamin B6: 0.14mg	Highest Mn content; Mn essential for MnSOD; chickpea peptides show ACE-inhibitory activity (antihypertensive)
Edamame (soybeans)	Vitamin K: 26µg; Folate: 311µg; Isoflavones: 40-60mg; Iron: 2.3mg; Complete protein	Only plant food with all essential amino acids in adequate proportions + isoflavones; isoflavone bioavailability best from whole soybean foods vs soy isolates
Kidney beans	Potassium: 405mg; Iron: 2.2mg; Thiamin: 0.16mg; Phytochemicals: coumestrol, kaempferol	DASH diet staple; hypoglycemic effect via amylase inhibition (phaseolamin) and viscous fiber

III. PROCESSING, COOKING & STORAGE — MICRONUTRIENT RETENTION

A. Effect of Different Processing Methods

Processing Method	Effect on Key Nutrients
Raw consumption	Maximum retention of vitamin C, folate, heat-labile B vitamins, and myrosinase (for glucosinolate → ITC conversion); however, cell walls limit carotenoid bioavailability; some anti-nutrients (oxalate, phytate) NOT reduced
Steaming (5-10 min)	Best overall cooking method: preserves ~70-90% vitamin C, ~70% folate, maintains myrosinase activity better than boiling; improves carotenoid bioavailability by softening cell walls without excessive leaching
Boiling (high water volume)	Significant nutrient leaching into cooking water: ~50-70% vitamin C loss, ~50-75% folate loss, ~60% water-soluble polyphenols; use minimal water or retain cooking liquid in soups/sauces; does improve lycopene and β -carotene bioavailability
Microwaving	Rapid, minimal water: excellent vitamin C retention (>90%); preserves polyphenols well; recommended for nutrient preservation
Stir-frying / Sautéing	Moderate heat, short time: good vitamin C retention (~75%); fat co-ingestion ↑ carotenoid and fat-soluble vitamin absorption; preferred for greens with olive oil
Roasting / Baking	High dry heat: significant vitamin C loss; creates Maillard browning products; concentrates minerals; carotenoid availability good (fat in dish enhances absorption); reduces anti-nutrients
Fermenting	Increases bioavailability of many B vitamins (microbial synthesis); reduces phytate (phytase) → ↑ mineral bioavailability; produces short-chain fatty acids; isoflavone deglycosylation ↑ absorption; creates probiotic microorganisms
Freezing	Blanching before freezing causes some nutrient loss but stabilizes remaining nutrients; frozen produce comparable or superior to 'fresh' stored produce in nutrient content (fresh produce loses ~30-50% vitamin C after 3-4 days storage)
Canning	Significant initial heat loss of vitamin C (50-80%) and folate; however, stable thereafter; lycopene in canned tomatoes notably more bioavailable; minerals and fiber fully retained

B. Storage Considerations

- Refrigeration: Slows enzymatic degradation; optimal for most F&V; however, some (tomatoes, bananas, tropical fruits) lose flavor compounds and texture with cold storage — store at room temperature

- Vitamin C degradation: Accelerated by light, oxygen, heat, and alkaline conditions; cut surfaces expose vitamin C to oxidation — minimize cutting ahead of time; store in airtight containers
- Folate: Highly sensitive to heat, UV light, and oxidation; cook minimally; do not store cooked greens for extended periods
- Ethylene gas: Produced by climacteric fruits (apples, bananas, pears) → accelerates ripening of nearby produce; store separately from vegetables to extend shelf life
- Practical takeaway: Fresh ≈ frozen > stored 'fresh' (for most vitamins); frozen F&V are nutritionally equivalent or superior to fresh produce stored for multiple days — a critical counseling point for cost-conscious and food-insecure patients

IV. F&V & CHRONIC DISEASE — EPIDEMIOLOGICAL EVIDENCE

A. Cardiovascular Disease

The cardioprotective effects of F&V are among the most consistently demonstrated associations in nutritional epidemiology:

- Aune et al. (2017, Lancet) meta-analysis of 95 prospective cohort studies (n>2.5 million): ≥5 servings F&V/day vs <1 serving → HR 0.80 for CVD mortality; 0.74 for ischemic heart disease; 0.81 for stroke
- DASH diet (Dietary Approaches to Stop Hypertension) clinical trials: Diet rich in F&V (8-10 servings/day) → SBP -3-6 mmHg, DBP -2-3 mmHg beyond control diet; magnified in hypertensive individuals; effect comparable to single antihypertensive medication in mild hypertension
- Potassium from F&V: Blunts sodium-driven hypertension (renal Na excretion); vasodilatory; reduces vascular stiffness; DASH diet provides ~4,700mg/day potassium vs US average ~2,600mg/day
- Dietary fiber from F&V: Soluble fiber (pectin, β-glucan) reduces LDL-C by 5-10% via bile acid sequestration, reducing hepatic cholesterol synthesis; FDA health claim for soluble fiber and reduced CHD risk
- Specific F&V with strongest CVD evidence: Green leafy vegetables (strongest inverse association with CVD in EPIC and Nurses' Health Study); berries; avocado (MUFAs + K + fiber); tomatoes (lycopene); cruciferous vegetables

B. Cancer

The World Cancer Research Fund (WCRF) Continuous Update Project and IARC provide the most rigorous F&V-cancer evidence synthesis:

- Non-starchy vegetables: Probable/convincing evidence for protection against oral/pharyngeal/laryngeal cancers, esophageal cancer (squamous), and stomach cancer
- Fruits: Probable evidence for protection against esophageal, lung, and stomach cancer; strongest associations for beta-carotene rich fruits and lung cancer (non-supplement)
- Cruciferous vegetables: Consistent inverse associations with bladder cancer, colorectal cancer, and lung cancer across cohort studies; glucosinolate/sulforaphane mechanism
- Allium vegetables: Inverse association with stomach cancer (strong — 6-fold higher gastric cancer in low allium consumption populations); anti-H. pylori and pro-apoptotic mechanisms

- Tomatoes/lycopene: Inverse association with prostate cancer (advanced/lethal stage); ~40% ↓ risk in highest lycopene quintile (Health Professionals Follow-up Study)
- Limitations: Heterogeneity in how F&V are classified, measured (24h recall vs FFQ), and confounded by overall healthy lifestyle; WCRF now focuses on overall dietary pattern rather than individual foods

Evidence: *WCRF 2018 Cancer Prevention Recommendations: Make whole grains, vegetables, fruits, and pulses (legumes) a major part of the usual daily diet. Aim for at least 30g dietary fiber per day. Limit consumption of 'fast foods' and processed foods (which displace F&V). No specific quantitative F&V intake target in updated guidelines due to evidence heterogeneity.*

C. Type 2 Diabetes

- Whole fruit (NOT juice): 3 servings/week of whole fruit (especially blueberries, grapes, apples, pears) → ~26% ↓ T2DM risk (Nurses' Health Study I & II + HPFS combined analysis, n>180,000); fruit juice associated with INCREASED risk (rapid glucose delivery, absent fiber)
- Leafy greens: Strong inverse association; 1.35 additional servings/day green leafy vegetables → 14% ↓ T2DM (meta-analysis, 6 prospective studies); magnesium, alpha-lipoic acid content, folate, polyphenols potentially contributing
- Cruciferous vegetables: Inverse association in EPIC and NHS cohorts; sulforaphane reduces hepatic glucose production and improves insulin sensitivity in diabetic models; clinical trial in T2DM patients: broccoli sprout extract significantly reduced fasting blood glucose and HbA1c vs placebo over 12 weeks
- Glycemic index nuance: Not all F&V are created equal; high GI fruits (watermelon, overripe banana, pineapple) eaten in moderation; low GI fruits (berries, apples, cherries, citrus) preferred for glycemic management; non-starchy vegetables universally low GI

Clinical Note: *F&V in T2DM management: Evidence supports non-starchy vegetables (unlimited) and 2-3 servings of low GI whole fruits daily even in diagnosed T2DM. The American Diabetes Association's Standards of Care emphasize non-starchy vegetables as foundation of the diabetes plate method. Restricting fruit entirely in T2DM is NOT evidence-based and deprives patients of important micronutrients.*

V. PRACTICAL DIETARY COUNSELING — INCREASING F&V INTAKE

A. Evidence-Based Behavior Change Strategies

- Make it visible and accessible: Wash and cut F&V immediately after shopping; place fruit bowl at eye level; pre-portion vegetables in clear containers in refrigerator — reduces preparation barrier
- Incorporate at every meal: Breakfast (berries/banana in oatmeal or yogurt; spinach in eggs/smoothie); Lunch (side salad, vegetable soup, raw vegetable platter); Dinner (half plate non-starchy vegetables as default)
- Frozen F&V parity: Nutritionally equivalent to fresh for most micronutrients; more affordable, longer shelf life, no waste — counsel food-insecure patients specifically that frozen is an excellent choice
- Canned F&V strategies: Choose no-added-salt canned vegetables; drain and rinse canned beans (removes ~40% sodium); canned tomatoes are nutritionally superior to out-of-season fresh for lycopene; canned fish (sardines, salmon) provides omega-3s + calcium from bones

- Cultural competency: Identify culturally traditional F&V and cooking methods; build recommendations around familiar foods; avoid prescribing unfamiliar foods that will not be incorporated; explore local markets, cultural grocery stores
- Cost reduction strategies: Seasonal F&V are 30-50% cheaper; farmers' markets may accept SNAP benefits; frozen and canned are cheaper than fresh; growing common herbs and vegetables at home (mint, tomatoes, green onions) is accessible even in small spaces

B. 'Eat the Rainbow' — Color-Based Counseling Framework

Color Category	Key Phytonutrients	Priority Sources
RED	Lycopene, anthocyanins, ellagic acid	Tomatoes, watermelon, strawberries, cherries, red peppers, pomegranate, red cabbage
ORANGE/YELLOW	Beta-carotene, alpha-carotene, vitamin C, hesperidin	Sweet potato, carrots, pumpkin, orange/yellow peppers, oranges, mango, papaya
GREEN	Sulforaphane, lutein/zeaxanthin, folate, vitamin K, isothiocyanates	Broccoli, spinach, kale, Brussels sprouts, edamame, avocado, kiwi, green tea
BLUE/PURPLE	Anthocyanins, resveratrol, pterostilbene	Blueberries, blackberries, purple grapes, eggplant, purple cabbage, beet, plum
WHITE/BROWN	Allicin, quercetin, kaempferol, inulin (prebiotic)	Garlic, onions, leeks, cauliflower, mushrooms, Jerusalem artichoke, white asparagus

Counseling Tip: Use the 'color goal' concept with patients: aim for at least 3 different color categories daily; use a simple tally card or phone photo log. Research shows color diversity correlates with phytochemical diversity and is a simple, memorable goal — more actionable than specifying grams or cups for many patients.

C. Special Population Considerations

Older Adults:

- Reduced gastric acid → impaired B12, Fe, Ca, and Zn absorption; F&V provide easily absorbed alternatives (plant calcium from low-oxalate sources, vitamin C to enhance Fe)
- Dysphagia or dentition issues: Soft-cooked, pureed, or smoothie-based F&V delivery; avoid raw hard vegetables; cooked fruit compotes; vegetable soups
- Drug interactions: Leafy greens (vitamin K1) with warfarin — emphasize consistency rather than avoidance; grapefruit with CYP3A4-metabolized drugs

Pregnant Women:

- Folate priority: 400-800µg/day periconceptionally; emphasize dark leafy greens, legumes, citrus; supplement in addition to dietary sources
- Vitamin C: Enhances non-heme iron absorption from plant sources — combine iron-rich legumes/greens with citrus or tomatoes at each meal
- Food safety: Avoid raw sprouts (Listeria, Salmonella risk); wash all F&V thoroughly; choose pasteurized juices

Vegans/Vegetarians:

- F&V are nutritional backbone; ensure variety across all color groups and categories
- Focus on calcium (bok choy, fortified plant milks, kale), iron (legumes + vitamin C enhancer), zinc (legumes, seeds, fermented soy), omega-3 (flaxseed, chia, walnuts for ALA; algal DHA supplement)
- B12: NOT found in any F&V; supplementation is mandatory for strict vegans regardless of F&V intake

Patients with CVD/Hypertension:

- DASH diet implementation: 8-10 servings F&V daily; emphasize K, Mg, and Ca-rich sources (bananas, sweet potato, leafy greens, legumes)
- Potassium caution in advanced CKD (eGFR <30): Restrict high-K F&V; leaching (boiling, discarding water) reduces potassium content ~50% — allows safe inclusion of vegetables in dialysis patients
- Sodium in canned: Drain and rinse, or choose no-added-salt products

Lecture 3 Summary — Key Principles

- Fruits and vegetables are nutritionally irreplaceable — providing vitamins (A, C, K, folate), minerals (K, Mg, Ca, Fe, Mn), fiber, and thousands of phytochemicals in synergistic combinations
- Micronutrient density champions: Dark leafy greens (K1, folate, lutein, Mg, Ca, Fe), cruciferous vegetables (C, K1, sulforaphane, folate), berries (anthocyanins, C, ellagitannins), legumes (folate, Fe, Zn, Mg, phytoestrogens)
- Cooking matters: Steam > microwave > stir-fry > boil for vitamin retention; cook tomatoes (lycopene ↑) + add fat; eat broccoli raw or lightly steamed (preserves myrosinase); frozen = fresh nutritionally
- CVD: 5+ servings F&V/day → ~20-26% ↓ CVD and stroke mortality (meta-analyses); DASH diet ↓ SBP comparable to antihypertensive medication
- Cancer: Cruciferous vegetables (bladder, colorectal), allium (gastric), lycopene (prostate), and overall non-starchy vegetable intake consistently associated with reduced cancer incidence
- T2DM: Whole fruit (NOT juice) reduces T2DM risk; leafy greens are particularly protective; non-starchy vegetables should form the dietary foundation for T2DM management
- Practical counseling: 'Eat the rainbow' (5 color categories), frozen = fresh, whole fruit over juice, build cultural competency, address access and cost barriers

Suggested Reading:

1. Aune D et al. (2017) Fruit and vegetable intake and the risk of CVD, total cancer, and all-cause mortality. *Int J Epidemiol.* |
2. WCRF/AICR Third Expert Report (2018). Diet, Nutrition, Physical Activity and Cancer: A Global Perspective. |
3. US Dietary Guidelines for Americans 2020-2025. |
4. Gunter MJ et al. (2017) Fruit and vegetable intake and cancer. *JAMA Oncol.*